THE ROLE OF DENITRIFICATION IN NITRATE REMOVAL FROM A POORLY DRAINED SANDY SOIL IRRIGATED WITH DAIRY EFFLUENT

By

SHOUCANG YAN

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To my wife Liang and my son Clayton

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By

Shoucang Yan

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Using dairy lagoon effluent for irrigation has become a common practice for dairy farms of Florida. The dairy farmers benefit both from proper disposal of the wastewater through irrigation and efficient nutrient recycling for crop production. However, this practice can potentially cause nitrate contamination of groundwater. Field and laboratory studies were conducted to determine (i) the effect of effluent irrigation on soil C, N and groundwater quality, (ii) the denitrification rate and its regulators in the soil profile, and the role of denitrification in protecting the groundwater from nitrate contamination, and (iii) the degree of carbon regulation for denitrification in both surface and subsurface soils. Effluent irrigation increased the NO_3^--N concentrations, exceeding the EPA drinking water standard of 10 mg NO_3^--N L^{-1} in the shallow groundwater. However, the

contamination was limited to a small area outside the irrigated field. The long residence time and biological denitrification in the groundwater appeared to be responsible for the nitrate removal. Irrigation with dairy effluent also increased the nitrate content of the soil profile. The nitrate produced through mineralization of organic N and nitrification was subject to rapid leaching in the highly permeable sandy soil.

Denitrification rates were high in the surface soil and decreased markedly with depth. Denitrification in the surface soil was primarily limited by aerobic conditions and, to a lesser extent, by available organic carbon. Denitrification in the subsoils were limited by the low population of viable denitrifiers resulting from a long-term shortage of available organic carbon. The dairy effluent provided a large quantity of organic carbon and therefore enhanced denitrification in the surface soil. The soil solution from effluent-treated surface soil also could promote denitrification in subsoils if transported to the greater depths in the soil profile. However, organic carbon from the effluent was generally decomposed in the soil profile before reaching the groundwater and, therefore, was less available to denitrifiers in the groundwater.

CHAPTER 1
INTRODUCTION

Denitrification is an anaerobic microbial process where nitrate is reduced first to nitrite and then to nitrogen gases which are commonly lost to the atmosphere. Denitrification is a key process in the biogeochemical nitrogen cycle, because it is the only mechanism that converts fixed nitrogen back to free dinitrogen form. Denitrification in the soil has important implications for both agricultural production and environmental quality. Most of the research on denitrification has been devoted to studying and minimizing its occurrence in agricultural soils in order to prevent nitrogen loss, and research in this area still remains a priority. However, denitrification has gained additional attention in recent years due to its role in affecting environmental quality. Nitrous oxide concentrations in the atmosphere have been increasing at 0.2 to 0.3% per year for last 30 years (Rasmussen and Khalil 1986). The increase of N_2O and other gases such as CH_4 and CO_2 is believed to be a major cause of global warming, and denitrification is one of the major contributors to atmospheric N_2O levels. There is also increased interest in denitrification because it is the only significant mechanism of nitrate removal in contaminated environments such as subsoils and aquifers. Denitrification has also been shown to play an important role in removing nitrate and protecting surface and groundwater resources in riparian ecosystems. The negative and positive implications of denitrification make it both a controversial biological process and an interesting research topic.

Statement of the Problem

Nitrate contamination of groundwater is widespread in the United States. Numerous studies have reported NO_3^--N levels in groundwater above 10 mg L^{-1}, the drinking water standard set by the World Health Organization and the U.S. Public Health Services. There are many local sources of nitrate that may contribute to the groundwater quality problem in an area. Over-fertilization and application of agricultural waste materials are among the most frequent nitrate contributors to groundwater in agricultural areas, however.

Using lagoon effluent for irrigation in agricultural soils is a common practice for disposing of wastewater produced on dairy farms in Florida. While providing nutrients for plant growth is essential, elevated NO_3^--N concentrations have been found in groundwater under many dairy operations (Andrews 1992).

The principal forms of N in dairy effluent are NH_4^+-N and organic-bound N. When applied to the soil surface, a series of N transformations take place in the soil profile. Any nitrate present in groundwater is the net result of nitrate production, transportation and consumption in the soil column. The two processes that determine the amount of nitrate reaching the groundwater are plant uptake and denitrification. While both processes may be active in the surface soil, the only significant NO_3^- removal mechanism below the root zone is denitrification.

Recently, studies have shown that significant denitrification activity could occur in some shallow groundwater systems (Starr and Gillham 1993, Trudell et al. 1986). Many studies have also found that denitrification plays an important role in nitrate removal in riparian soils, which serve as nutrient buffer zones for protecting both groundwater and surface resources (Jacobs and Gilliam 1985, Lowrance et al 1985). However, results regarding denitrification in subsurface soil and riparian zones are divergent. Therefore, the regulation of this process in subsurface environment remains largely unknown and research in this area is urgently needed.

Research Objectives

The overall goal of this study was to evaluate the effect of dairy lagoon effluent irrigation on groundwater beneath a landscape containing a sprayfield and an adjacent riparian zone. The study site provided a unique landscape, with different landforms in a small area and nitrate impacted shallow groundwater flowing from the field towards a forested wetland along a hydrologic gradient.

To evaluate the impact of dairy effluent irrigation on groundwater quality in this particular unconfined shallow aquifer, and to study regulation of the nitrate removal process, denitrification, in the soil profile, the following questions were addressed:

(1) What impact does the effluent irrigation have on groundwater quality?

(2) What is the extent of the impact and its relation to groundwater hydrology?

(3) What is the effect of effluent irrigation on soil chemistry?

(4) What is the denitrification rate in this system and what are the major regulators in the soil profile?

(5) What role does denitrification play in protecting groundwater from NO_3^- contamination in this area?

(6) What is the role of organic carbon in regulating denitrification in surface and subsurface soils?

Dissertation Format

The first two chapters of this dissertation consist of a general introduction and literature review. The subsequent chapters are written as independent manuscripts intended for publication. Chapter 3 examines the impact of dairy effluent irrigation on groundwater chemistry. The effect of effluent irrigation on soil chemistry and the role of the soil column in regulating denitrification are also discussed in chapter 3. In chapter 4, ambient and anaerobic denitrification rates are reported and the major regulators of denitrification in different zones and in the soil profile are reported. The important role of organic carbon in regulating denitrification is presented in chapter 5, with a summary and conclusions being reported in chapter 6.

CHAPTER 2
LITERATURE REVIEW

Introduction

Denitrification plays a central role in the overall nitrogen cycle. Improvements in measurement technology have generated a considerable amount of research activity on denitrification in the last few decades; however, it still remains one of the poorly understood facets of the soil nitrogen cycle (Betlach and Tiedje 1981). This is partially because denitrification is a microbial process that is highly regulated by many environmental factors, with these factors varying greatly from system to system. This chapter will review some of the basic knowledge of denitrification including microbiology, study methods, and environmental regulators. Since increasing attention has been given to nitrate pollution of groundwater resources in recent years, information about denitrification in subsurface environments, and its regulation and role in protecting groundwater, will also be reviewed.

Definitions

Denitrification is an anaerobic microbial process whereby nitrate, serving as an electron acceptor during biological cell metabolism, is reduced to nitrite and then to gaseous nitrous oxide and dinitrogen which are commonly lost to the atmosphere (Knowles 1982). Denitrification is not the only nitrate-reduction process occurring in the soil, however. Denitrification must be distinguished from assimilatory reduction of nitrate and from dissimilatory nitrate reduction of nitrate to ammonium. Although these are all nitrate reduction processes, the pathways, participating organisms, and environmental regulators are different. The differences among these biological nitrate reduction processes are summarized in Table 2-1.

Microbial denitrification also is not the only process yielding N_2O and N_2 gases. These gases can be produced by a nonenzymatic pathway under fully aerobic conditions. This N_2 generating process, catalyzed by abiotic agents, is called chemodenitrification (Verdegem and Baert 1984). Although chemodenitrification does occur, it is not a major process on a global scale (Tiedje 1988).

Denitrification is a respiratory process because reduction of the nitrogen oxide is coupled to electron transport phosphorylation, and energy can be conserved by the reduction process. There are also some nonrespiratory denitrifiers which produce N_2O but not N_2 as reduction products. Its significance has been demonstrated in some forest soils (Tiedje 1988). Nitrification also has been shown to produce N_2O (Robertson and Tiedje 1987).

Table 2-1. Nitrate reduction mechanisms (After Tiedje 1988)

Mechanism	Pathway	Regulator	Participant
Assimilatory nitrate reduction	$NO_3^- \to NO_2^- \to NH_4^+ \to$ org-N	NH_4^+-N org-N	Plants fungi algae bacteria
Denitrification	$NO_3^- \to NO_2^- \to N_2O \to N_2$	O_2	Facultative bacteria
Dissimilatory nitrate reduction to ammonium	$NO_3^- \to NO_2^- \to NH_4^+$	O_2	Anaerobic and facultative anaerobic bacteria

Denitrifying Bacteria

A wide range of physiologic and taxonomic groups of bacteria are capable of denitrification in soil. The main genera of denitrifying bacteria commonly isolated from soil include *Alcaligenes, Bacillus, Flavobacterium, Paracoccus* and *Pseudomonas* (Betlach and Tiedje 1981, Tiedje 1988, Verdegem and Baert 1984).

Although denitrification only occurs under anaerobic conditions and when nitrate is present, most denitrifying bacteria are facultative aerobic organisms. Therefore, their existence in nature is based on their ability to compete for carbon sources with aerobic organisms, rather than on their denitrification properties (Tiedje 1988). The facultative nature of denitrifying bacteria make them compatible with either obligate aerobes or obligate anaerobes; therefore, they are present in nearly all soils.

Most denitrifying bacteria in soil are heterotrophic; i.e. they use organic carbon as an electron donor during their respiration. There are also some autotrophic denitrifiers that can use reduced inorganic compounds such as Fe^{+2}, Mn^{+2} and HS^- as electron donors. Autotrophic denitrifiers have been found in some environments, such as below the root zone in agricultural soils (Verdegem and Baert 1985) and in ground water (Lind 1983).

Among the denitrifying bacteria, only one phototrophic denitrifier, *Rhodopseudomonas*, and one spore-forming species of *Bacillus* have been reported (Tiedje 1982).

Most denitrifiers can not ferment, and therefore can not survive under anaerobic conditions without nitrate. However, some bacteria have been found to carry the properties of both denitrification and fermentation, e.g. *Propionbacterium acidi-propionici* and *Bacillus spp*. (Tiedje 1982).

Methods for Studying Denitrification

Denitrification rate is the most commonly measured parameter for studying denitrification processes. The rate measurement can be used to study the amount, rate, and timing of nitrogen loss in a system. There are several methods available for measuring denitrification rate at both laboratory and field scales. The commonly used methods include indirect methods such as nitrate disappearance, nitrate/chloride ratio, nitrogen balance, and direct measurement of the denitrification products, N_2O, and N_2, by using various sampling and analytical approaches including ^{15}N isotope tracer technique (Hauck 1986).

Nitrate Disappearance

Denitrification rate can be inferred by monitoring the disappearance of nitrate nitrogen in soil. This is the simplest method ,and is mainly used in confined situations where the major losses of nitrate is via denitrification.

However, assimilation of nitrate to organic N and dissimilatory reduction of nitrate to ammonium also occur in some soils under certain conditions. Tiedje et al. (1982) showed that dissimilatory nitrate reduction to ammonium can compete with denitrification for available nitrate in some natural systems, with such competition depending on whether nitrate or organic carbon was limiting in the system (Tiedje et al. 1982). The problem of not distinguishing the fate of nitrate can be overcome by using ^{15}N-labeled nitrate, where the extent of nitrate immobilization or reduction to ammonium can be determined concurrently (Hauck 1986).

Nitrate to Chloride Ratio

Use of nitrate to chloride ratios to determine any losses of nitrate nitrogen is based on the assumption that chloride moves with nitrate in soil at the same rate, without unaccountably being removed from solution (Hauck 1986). However, due to the high spatial variability of both ions in soils, this method has limited use in the field. It works well in controlled laboratory column studies, however, where nitrate and chloride concentrations are known. In spite of the limitations, this method has been used to qualitatively determine relative nitrate losses in soils (Jacobs and Gilliam 1985, Schipper et al. 1989,).

^{15}N Isotope Technique

Since the ^{15}N isotope technique was first introduced by Hauck and Bouldin (1961) and Hauck et al. (1958) to study denitrification in soil, this method has been extensively used in field and laboratory studies because of its high sensitivity. There are different approaches when using the ^{15}N technique including the ^{15}N balance approach, direct measurement of $^{15}N_2$ and $^{15}N_2O$ evolution, and the ^{15}N isotope dilution technique (Aulakh et al. 1992, Myrold 1990). The reader is referred to the following references regarding basic principles (Aulakh et al. 1992, Myrold 1990), application in field studies (Aulakh et al. 1992), and comparison of acetylene inhibition and ^{15}N techniques (Hauck 1986, Myrold 1990).

The most commonly used approach when using the ^{15}N technique is direct measurement of $^{15}N_2$ and $^{15}N_2O$ evolution. This involves applying a ^{15}N-enriched N source to the soil, followed by sampling and analyzing the nitrogen gases evolved into a confined environment. The rate of change of ^{15}N atoms in the gas phase over time can be determined using isotope ratio mass spectrometry (Hauck et al. 1958).

Use of the ^{15}N technique is based on several assumptions. One major assumption made in field applications is that the ^{15}N nitrate labeled material can be assumed to exist in a single pool that is isotopically uniform (Warwick and Hill 1988). This has been found not to be the case, however, due to spatial variability in the relative amount of soil- and fertilizer-derived nitrate (Mosier et al. 1986, Mulvaney 1988).

Radioactive tracer (^{13}N) techniques have also received considerable attention in recent years. The major advantage of this technique is its high sensitivity. The $^{13}N_2$ can be measured directly under most atmospheric conditions, which is not possible using ^{15}N techniques. However, the very short half life of ^{13}N limits its application, especially for field studies. Despite these limitations, this technique has been used by several researchers in studying denitrification (Smith et al. 1978, Tiedje et al. 1978, Tiedje et al. 1979, Tiedje et al. 1981).

Acetylene Inhibition Method

Among the several methods available, the acetylene inhibition technique has received the most attention in the last three decades. The observation that N_2O reduction to N_2 during denitrification can be inhibited by acetylene at 0.01 atm (Balderston et al. 1976, Yoshinari and Knowels 1976) formed the basis of the acetylene inhibition method. The method simply involves treating the soil atmosphere with 1-10 KPa acetylene, followed by sampling and analyzing the N_2O evolved using gas chromatography (Klemedtsson et al. 1990). Since the reduction of N_2O to N_2 is blocked, the amount of N_2O measured represents the total potential production of

N_2O and N_2. The advantages of this method include its relatively simple and straight-forward approach, relatively inexpensive instrumentation, low detection limit, and the fact that the method is not destructive.

There are two commonly used approaches for the acetylene inhibition method: the chamber and soil core methods. In the chamber method, acetylene is injected into the soil at certain depths using a diffusion probe. The acetylene can be recirculated continuously (Aulakh et al. 1991), or can be injected at one time (Mosier et al. 1986). Gas samples are taken from the chamber inserted into the soil. Although the amount of acetylene used varies from soil to soil, it has been found that acetylene concentrations of > 0.1% in the soil atmosphere are adequate for complete inhibition of reduction of N_2O to N_2 (Duxbury 1986, Yoshinari and Knowels 1977).

The intact soil core method is another commonly used approach. An intact soil core can be taken by pushing a metal probe into soil. The soil core is then incubated in a container to which acetylene is added. Either plastic tubes, airtight jars or serum bottles can be used for the incubation. A rubber septum is required to allow for easy gas sampling (Tiedje et al. 1989).

By comparing four different field methods for measuring denitrification, Aulakh et al. (1991) fond that chamber methods either using ^{15}N tracer or acetylene inhibition were reliable but that proper sampling intervals had to be chosen. They also found that results given by the intact soil core method were comparable with those measured by the chamber methods.

The acetylene inhibition method has several limitations which must be avoided when this method is applied in a field study. The major limitation is that the acetylene also inhibits nitrification (Mosier 1980). Therefore, in those systems where nitrate supply is limiting, the concurrent inhibition of nitrification will result in underestimation of denitrification rates. Secondly, the acetylene may undergo microbial decomposition in soil (Terry and Duxbury 1985, Yeomans and Beauchamp 1982). Yeoman and Beauchamp (1982) reported an increase in production of N_2O and N_2 gases in the absence of carbon substrate when acetylene was used. In addition, adaptation of organisms to the acetylene environment is possible. Yeomans and Beauchamp (1978) found that microorganisms were able to reduce N_2O in the presence of acetylene; therefore, the method can only be used for short-term studies. Lastly, uniform distribution of acetylene must be ensured before actual measurements are made. Although acetylene has high water solubility, an adequate distribution in clay and poorly drained soils is difficult to achieve. The same is true for deep soil-profile treatments (Rolston 1986). Because of the limitations discussed above, a preliminary study should be conducted to investigate the optimum protocol suitable to the specific site studied.

Factors Affecting Denitrification

As with other nitrogen transformation processes, denitrification is affected by many environmental factors. Denitrification can only occur when the right conditions exist (Davidson et al. 1990). The main factors controlling denitrification include oxygen and nitrate concentration, pH, and temperature. Each of these factors is, in turn, affected by other factors such as soil water content, the presence of plants and soil texture. This makes it difficult to study denitrification by just examining one parameter. To simplify this complexity, Tiedje (1988) proposed a two-dimensional conceptual model for the environmental regulation of denitrification (Fig. 2-1). In this model, the environmental factors are divided into two groups. The first group is called proximate factors, including oxygen, nitrate, and carbon. This group of factors exert direct influence on denitrification at the cellular level. The second group is called distal factors, including soil water, plant, soil texture, soil respiration, soil organic matter, etc., which control the proximate factors at a larger scale. It can be seen from the model that each of the proximate factors may be affected in turn by several distal factors.

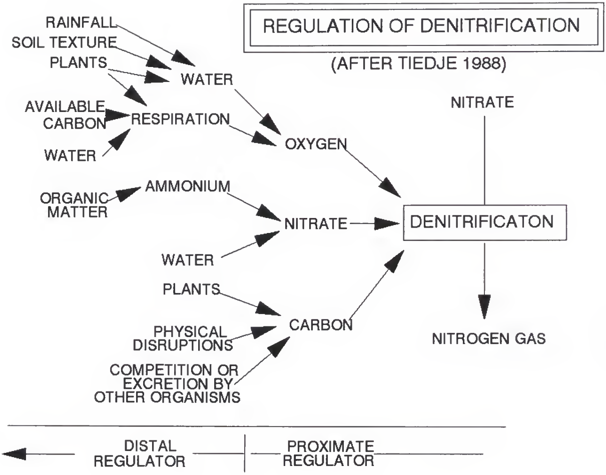

Fig. 2-1. Factors regulating denitrification in the soil environment.

Soil water and organic matter are the common distal factors that influence all three proximate factors.

The model also shows the relative importance of each of three proximate factors. Oxygen is the major regulator of denitrification because both synthesis and activity of the denitrifying enzymes are inhibited by oxygen (Tiedje 1988), even at a very low concentration of 0.05% O_2 (Groffman et al. 1988). In order for denitrification to occur, there must be essentially depletion of oxygen. Since nitrate serves as an electron acceptor, its supply certainly affects the rate of denitrification in systems where nitrate is limiting the process. The rate of denitrification can also be limited by the availability of organic carbon.

Oxygen

Oxygen is the dominant regulator of denitrification due to its direct inhibition of the process. Its regulatory role has been illustrated by many studies in both pure culture and natural soil systems. Work by Parkin and Tiedje (1984) showed that the denitrification rate under aerobic conditions is generally 0.3-3% of that under anaerobic conditions. Although aerobic denitrification appears at times to occur, it is generally agreed that anaerobic microsites are responsible for what appears to be aerobic denitrification.

The threshold value of oxygen concentration permitting denitrification to occur has been studied (Tiedje 1988). From the data reported, mostly from aquatic system and pure culture, Tiedje (1988) concluded that 10 umol/l or less of oxygen is required to initiate denitrification. It is noteworthy that these data were collected from different studies under a variety of conditions. The units used to express the concentration were often also different as well. Therefore, the data should be carefully evaluated when such a comparison is made.

Trevors (1985) reported that denitrification was not observed until the oxygen concentration decreased to 0.2 and 0.28 μmol L^{-1} in the soil and gas phase, respectively. By using the soil core method, Parkin and Tiedje (1984) found that denitrification rate increased greatly at oxygen pore-space concentrations below 0.5%. Relating oxygen to soil water content, several studies have suggested that denitrification is negligible at water contents below 60% of maximum water holding capacity (Aulakh et al. 1992).

The biological mechanism for denitrification inhibition by oxygen is not clear, but it has been shown that oxygen affects both synthesis and activity of denitrifying enzymes (Tiedje 1988). Furthermore, the later reductase (i.e. nitrous oxide reductase) is more sensitive to oxygen than the early reductase (Betlach and Tiedje 1981, Tiedje 1988). It has been suggested that a "semianaerobic condition" is necessary for synthesis of at least nitrate reductase (Calder et al. 1980, Payne et al. 1971).

The oxygen status of a soil microsite is regulated both by rate of supply and rate of consumption. Many factors are involved in controlling these two rates. Anaerobiosis results when the rate of consumption exceeds the rate of supply. The supply of oxygen to microorganisms in soil is mainly maintained by diffusion of oxygen through the soil pores. The oxygen diffusion rate through water is approximately 10^4 times slower than through air, which greatly restricts the diffusion of oxygen. Therefore, denitrification tends to be reversely related to the soil water content.

Rainfall and irrigation are the major water sources for soil, and the pattern and rate of rainfall and irrigation events greatly affect the extent of denitrification. A rapid increase in denitrification rate after rainfall and irrigation has been reported by several researchers for different soils (Rolston et al. 1982, Sexstone et al. 1985).

Oxygen concentration is also affected by soil texture. This effect results mainly from soil structure, water infiltration rate, and water holding capacity. These, in turn, directly affect oxygen transport in porous media (Aulakh et al. 1992). Chaterpaul et al. (1980) found that denitrification rate increased with increase in fineness of the soil in the order: clay loam > loam > sandy loam.

Groffman et al. (1989a, 1989b) used soil texture as a general predictor of denitrification activity at the landscape scale in forest soils.

The major sinks (consumption) of oxygen in soil are plant root and microbial respiration, which are driven in turn by supply of available organic carbon. Addition of organic materials such as manure (Guenzi et al. 1978), sludge (Lance 1986), straw (Aulakh and Rennie 1987), and plant residues (Aulakh et al. 1984) has been shown to increase the denitrification rates of aerobic soils. Organic carbon influences denitrification by both directly supplying carbon sources for microbial growth, and indirectly through the consumption of oxygen by other microorganisms.

Nitrate

Nitrate concentration directly affects the denitrification process. Much like oxygen, the amount of nitrate available to denitrifying bacteria is controlled by production, consumption and transport in soil (Groffman et al. 1988).

Except for fertilized agricultural soils, the production of nitrate in most natural systems is via autotrophic and heterotrophic nitrification under aerobic conditions (Davidson et al. 1990). Therefore, any factor affecting nitrification will influence the amount of nitrate available for denitrification. Since ammonium, the substrate of nitrification, is produced during the decomposition of organic matter, the organic matter mineralization rate is often a dominant factor affecting nitrification (Robertson 1982).

Nitrification normally occurs in an aerobic environment, but denitrification only takes place in anaerobic zones or at anaerobic microsites; therefore, the diffusion of nitrate from aerobic to anaerobic zones often controls the rate of denitrification. This has been verified in both upland and wetland soils. Myrold and Tiedje (1985) found that nitrate diffusion limitations occur when the mean aggregate radius was greater than 2 mm, and they concluded that diffusion limitation existed in most aggregated soils under aerobic conditions. Tiedje (1988) also concluded that diffusion of nitrate often limited denitrification in both unfertilized agricultural and forest soils. Diffusion of nitrate plays a role as well in controlling denitrification in wetland soils, where the aerobic and anaerobic zones tend to be stratified in the vertical soil profile. The nitrate produced in the aerobic layer has to diffuse downward to the anaerobic zone, where denitrification take place (Philips et al. 1978, Reddy et al. 1978).

Nitrate can be consumed by microbial assimilation, denitrification, dissimilatory nitrate reduction to ammonium, plant uptake, and leaching losses (Tiedje 1988). The nitrate available to denitrifiers is actually based on the competition of denitrifiers with other nitrate consumers. In unfertilized agricultural soils, plant uptake could be an important competing factor (Smith and Tiedje 1979a); however, this is unlikely the case under anaerobic conditions (Christensen and Tiedje 1988). Microbial assimilation could also be an important sink for nitrate (Schimel et al. 1989).

The effect of nitrate concentration on denitrification generally follows a Michaelis-Menten relationship. First-order kinetics will be followed at low nitrate concentrations, whereas zero-order kinetics control the denitrification rate at high nitrate concentrations (Knowles 1982). The critical concentrations of nitrate for the change of kinetic rates varies greatly (Aulakh et al. 1992, Betlach and Tiedje 1981). Tiedje (1988) attributed these different kinetics to the effects of diffusion along with different affinities that denitrifiers have to the nitrate in natural systems. In fact, interaction of the factors in nature makes it difficult to study the kinetics of denitrification in natural systems and often makes the existing kinetic constants less meaningful (Aulakh et al. 1992). Bowman and Focht (1974) and Kohl et al. (1976) suggested that availability of carbon must be taken into consideration when the kinetics of denitrification are studied. Reddy et al. (1978) and Philips et al. (1978) also concluded that nitrate diffusion was more important than nitrate reduction in controlling denitrification kinetics.

Soil pH

Like most heterotrophic microorganisms, denitrifying bacteria have optimum growth at nearly neutral pH (ranging from 6 to 8)(Aulskh et al. 1992, Betlach and Tiedje 1981). Within this range, however, the pH has little effect on denitrification rate (Burford and Bremner 1975, Stanford et al. 1975). Although denitrification is favored at slightly alkaline pH, few studies have been reported on the effects of high pH on denitrification. Studies on pH effects show that denitrification can occur as low as pH 3.5 (Gilliam and Gambrell 1978, Parkin et al. 1985,). However, denitrification activity is inhibited in acidic natural systems. Klemedtsson et al. (1978) found that a pH of 3.5 limited denitrification in a peat soil. They did not observe an increase in denitrification rate after addition of carbon and nutrients, though an increase of pH to 6.5 strongly stimulated denitrification. Parkin et al. (1985) suggested that long-term acidic soil conditions appear to select for a denitrifying population adapted to the low pH, even though they could not isolate the acid tolerant denitrifiers from the soil.

How pH affects denitrification is not clear, although there is some speculation. Koskinen and Keeney (1982) reported that pH affected denitrification by controlling the availability of carbon to denitrifiers. In a review by Firestone (1982), she speculated that, at low pH, nitrite could be toxic along with the aluminum and manganese solubilized at low pH. It also has been reported that low pH increases the mole fraction of nitrous oxide in the products of denitrification (Blackmer and Bremner 1978, Firestone et al. 1980, Weier and Gilliam 1986).

Temperature

The effect of temperature on denitrification , under ideal conditions, should obey the Arrhenius equation:

$$\ln v = (- H^{*}/RT) + C \qquad \text{(Firestone 1982)}$$

where v is the velocity, H^{*} is the activation energy, R is the gas constant, T is the temperature in ^{0}K, and C is a constant. Within the limit of enzyme activity, the denitrification rate increases exponentially with increase in temperature (Firestone 1982). The minimum, maximum and optimum temperatures vary from study to study, with the minimum temperature at which denitrification occurs ranging from -4°C to 10°C (Firestone 1982, Malhi et al. 1990). Denitrification could occur at temperatures as high as 75°C (Keeney et al. 1979). The optimum temperature has been reported to be approximately 65°C (Bremner and Shaw 1958, Malhi et al. 1990). The presence of thermophilic *Bacillus* species was thought to be responsible for this high optimum denitrification temperature (Paul and Clark 1989). Above 50°C, chemical decomposition of nitrite may also become significant (Keeney et al. 1979).

From an extensive study of both temperate and tropical soils, Gamble et al. (1977) suggested that denitrifying bacteria were not only capable of growing, but also adapted to growth, over a wide range of soil temperatures. The lower optimum temperature of 40°C found in a Canadian soil by Malhi et al. (1990) suggested that denitrifiers had adapted to the cold soil climate.

Organic Carbon

Organic carbon is one of the most important regulators controlling denitrification (Beauchamp et al. 1989). It is well-known that denitrifiers depend strongly on carbon as an electron donor as well as for cellular materials for their growth. Organic carbon also indirectly affects denitrification by consuming and depleting oxygen through aerobic respiration in soil, thereby enhancing the potential for denitrification.

Carbon limitations

In most natural systems, since denitrifying bacteria only account for a few percent of the total heterotrophic microbial population, the amount of carbon they can process is small (Tiedje 1988). Under anaerobic conditions, the competition of obligate aerobes no longer exist; therefore, carbon is less likely to be limiting for denitrification process (Reddy et al. 1982). However, there are some circumstances where limitation of carbon does occur. Sexstone et al. (1985) noticed a pulse increase in denitrification rate after rainfall; however, this increase was not observed for later rainfall. They attributed this to the depletion of carbon, since nitrate was still present. Low organic carbon availability has also been found to limit denitrification at lower depths in the soil profile (Aulakh et al. 1983, Parkin and Meisinger 1989).

Major controllers of organic carbon in soil

The major controllers of organic carbon status are given in the model proposed by Tiedje (1988). Water is the transport media for dissolved organic carbon and also regulates other denitrification factors. Plants constitute the major carbon source for the soil environment, and decomposition of plant materials provides the majority of the soil carbon pool. Living plant roots also excrete readily available carbon and influence both soil structure and oxygen content. Physical disturbance can release the protected carbon and enhance the accessibility to denitrifiers. Competition with plant roots and other microorganisms may play an important role under some conditions. Organic matter amendments, including addition of plant residues, manure, and waste water, can greatly influence the soil organic carbon balance.

Carbon sources for denitrification

Although denitrifying bacteria can use a wide range of organic acids, carbohydrates, and aromatic compounds under aerobic conditions, the number of compounds that can be used under anaerobic conditions is quite limited (Beauchamp et al. 1989). In order to test the effect of carbon on denitrification, many researchers use pure compounds such as glucose, sucrose, mannitol, and citrate (Bremner and Shaw 1958), acetate, propionate, and butyrate (Paul et al. 1989). Some of these compounds are used because they are the products of anaerobic decomposition (Paul et al. 1989). The efficiency of these compounds to stimulate denitrification differs. After comparing glucose, sucrose, mannitol and citrate as carbon sources, Bremner and Shaw (1958) found that they were all efficient in stimulating denitrification and concluded that available carbon should be both easily decomposed and able to support microbial growth. Paul et al. (1989) found that the quality of different compounds depended on the amount of electrons available per mole of carbon. They found that the denitrification capacity per mole of carbon differed in the order: sucrose < glucose < acetate < propionate < butyrate.

Other complex carbon sources have also been used to stimulate denitrification. It has been found that incorporation of manure (Guenzi et al. 1978) and plant residues (Aulakh et al. 1984, Aulakh et al. 1992, deCatanzara and Beauchamp 1985) increased denitrification; however, the response was complex. The quality of the material is more important than its quantity. The C/N ratio of the material has been found to be important factor affecting denitrification (Aulakh et al. 1991).

Denitrifying bacteria may also depend on other microorganisms to decompose the raw materials first and make the carbon more readily available. Paul and Beauchamp (1989a) found that denitrification and fermentation occurred simultaneously during anaerobic degradation of plant material. In another set of experiments, they observed a good correlation between denitrification and the disappearance of volatile fatty acids which are common fermentation products (Paul and Beauchamp 1989b).

Carbon availability for denitrification

Due to the complexity of organic matter in the soil environment, available carbon has not been precisely defined with respect to molecular size, molecular bonding and solubility. Davidson et al. (1987) defined available carbon as "organic carbon which exists in forms readily utilizable as an energy and carbon source by heterotrophic microorganisms". However, there is no single standard method for measuring carbon availability.

A basic approach to assess the availability of organic carbon is to evaluate the degree of correlation between denitrification and the amount of carbon measured by a given method. The commonly used methods for measuring the availability of organic carbon are listed in Table 2-2.

These methods can be basically divided into two groups: 1) extraction of organic carbon using selected reagents, and 2) measurement of decomposition of organic matter under defined conditions. Their use in the past greatly enhanced understanding of the availability of organic carbon, although compatibility among these methods still remains problematic.

Among the earliest studies on available carbon, Bremner and Shaw (1958) observed a general relationship between soil organic matter and denitrification. They found that easily decomposable substances such as glucose, mannitol and sucrose had greater effects than difficultly decomposable substances such as lignin and sawdust. This suggested that denitrification in soil was controlled largely by the supply of water-soluble or readily-decomposable organic carbon.

By studying the relationship between denitrification and available carbon using 17 surface soils, Burford and Bremner (1975) found that water-soluble and mineralizable carbon better correlated with denitrification than total

Table 2-2. Methods for assessing available organic carbon.

Method	Reference
Cold water soluble	Davidson et al. 1987, Burford and Bremner 1975
Boiling water soluble	Davidson et al. 1987
Mineralizable: aerobic	Davidson et al. 1987
Mineralizable: anaerobic	Davidson et al. 1987
Total organic carbon	Bijay-Singh et al. 1988
0.1 N $Ba(OH)_2$-extractable	Davidson et al. 1987
0.5 M K_2SO_4-extractable	Beauchamp et al. 1980
Microbial biomass	Drury et al. 1991

organic carbon. However, Beauchamp et al. (1980) studied several soils with different texture and drainage classes involving A, B, and C horizons. They found that total organic carbon gave the highest correlation with denitrification; water-soluble, mineralizable and 0.1 N $Ba(OH)_2$-extractable carbon showed less satisfactory correlation. They also observed that, while all methods were satisfactory for well drained soil, only total organic carbon and 0.1 N $Ba(OH)_2$ extractable carbon were satisfactory for poorly drained classes. They did not explain but this suggests that, when water soluble organic carbon is used as indication of available carbon, soil texture and/or drainage should be taken into consideration.

More recently, Davidson et al. (1987) tested and compared several methods for assessing available carbon including cold water extractable, boiling water extractable, mineralizable and total organic carbon. They concluded that mineralizable carbon under aerobic conditions provided the best measurement of available carbon. They based their judgement on: 1) the belief that measured carbon should reflect the carbon pool actually available to heterotrophic microorganisms; 2) performance of the procedure in predicting carbon limited process situations; and 3) the net effect of convenience, simplicity and cost.

Measuring water soluble carbon (WSC) makes ecological sense, since water is the medium by which carbon is transferred from one pool to another, and it is also generally assumed that organic carbon has to be water soluble before it can be utilized by microorganisms (Zsolnay and Steindl 1991). However, water soluble carbon only indicates what is present in the water phase, and does not necessarily reflect the relative ease with which microorganisms can attack this portion of organic carbon under natural conditions. Some researchers have found good correlations between water soluble carbon and denitrification rates, however (Burford and Bremner 1975, Davidson et al. 1987).

Whereas the WSC indicates the potential available carbon pool, mineralizable carbon presumably measures all carbon that has been mineralized by denitrifiers. This makes the method an appealing procedure, because it is the only mean for measuring the carbon that is really available for denitrification (assuming operationally that what has been used is that portion which is available).

Mineralizable carbon can be measured under both aerobic and anaerobic conditions. Burford and Bremner (1975) found a high correlation between denitrification rate and aerobically mineralizable carbon. Good correlations were also observed by Davidson et al. (1987) and Bijay-Singh et al. (1988), who studied a wide range of agricultural and desert soils. In all cases, mineralizable carbon under aerobic conditions was higher than WSC content, which suggests once more that denitrifying bacteria can utilize more carbon than WSC can provide.

The mineralizable carbon under anaerobic conditions has been found to be a good indicator of available carbon for denitrification. Christensen (1992) studied the decomposability of recalcitrant soil carbon during denitrification, and concluded that denitrification with excess nitrate was the easiest and most reliable procedure for testing the bioavailability of soil carbon. Bijay-Singh et al. (1988) found that, for field moist soils, the denitrification rate showed closest correlation with anaerobically mineralizable carbon under nitrate non-limiting conditions. Both studies suggested that the best estimation of carbon available to denitrifiers is made under conditions similar to those under which denitrification occurs. It is noteworthy, however, that the procedure used by previous authors was actually measuring the maximum carbon the denitrifiers can utilize, since nitrate was not limiting.

It should be realized that, although a good correlation can be found when one is studying the effect of water soluble carbon on denitrification, almost nothing is known about the composition of the water (or other reagent) extractable carbon pool. By theoretically calculating the amount of available carbon required for denitrification, Burford and Bremner (1975) found that 28% of the carbon used for denitrification had to be obtained from water insoluble form. This further suggests that water soluble carbon is only part of the carbon source for denitrification. Davidson et al. (1987) came to a similar conclusion, that cold water extractable carbon was certainly available to denitrifiers but was only part of the carbon pool that denitrifiers can utilize. On the other hand, evidence has also shown that not even all of the water soluble carbon is available to denitrifiers. Beauchamp et al. (1989), based on an analysis of published data, speculated that a significant amount of water soluble carbon seemed to be unavailable to microorganisms. Reddy et al. (1982) found that approximately 25% of the water extractable carbon was available apparently only at a relatively slow rate.

Because of the uncertainties about water- and other reagent-extractable carbon, another logical approach would be the further study of the composition of different pools of organic carbon. Isolation of specific compounds is at present difficult or impossible. A fractionation scheme is therefore desirable to fractionate the organic carbon into different groupings so that availability of carbon in each group can be tested subsequently. Unfortunately, no ideal method for such fractionation exists. Cook and Allan (1992) fractionated dissolved organic carbon from soils into 6 groups: hydrophilic acids, bases, and neutrals; and hydrophobic acids, bases, and neutrals. However, they were not able to detect differing changes among the different fractions during subsequent incubation. They concluded that this scheme was not able to distinguish the labile from the unavailable portion of the dissolved organic carbon.

The decomposition of plant residues, manure, and microbial biomass is the ultimate source of organic carbon for denitrifying bacteria. However, the distribution of organic carbon in the soil is affected by factors such as soil water movement, temperature, plant growth etc. Study of the effect of organic carbon on denitrification has been mainly conducted on surface soils. The dynamics of organic carbon in the lower profile, and its role in denitrification, is less studied. Meek et al. (1974) found increased quantities of soluble carbon as far as 140 cm below the soil surface after application of manure to an irrigated desert soil. They concluded that the high levels of water soluble carbon increased denitrification at greater depths in the soil profile.

In a conceptual model for the flow of organic materials through a soil , Zsolnay and Steindl (1991) separated mobile organic material from the immobile fraction. Water extractable organic carbon has interactions with both mobile and immobile phases. They also suggested that only the mobile organic materials could move down to lower soil depths. Meek et al. (1974) found increased denitrification rates in lower soil profiles after irrigation of a desert soil with manure effluent. All these findings suggest that the transport of organic carbon to the lower soil depths plays an important role in regulating denitrification in such zones.

Autotrophic Denitrification

Most denitrifiers are heterotrophic bacteria (Payne (1981) that use organic carbon as both a carbon and energy source (an electron donors). However, some bacteria can reduce nitrate to nitrogen gas by oxidizing a variety of reduced compounds including Fe^{2+}, Mn^{2+}, and HS^-. Nitrate serves as an electron acceptor and the reduced species as electron donors, and thermodynamic considerations confirm that this is possible (Korom 1992). This is called autotrophic denitrification because these microorganisms use inorganic carbon instead of organic carbon as their source of carbon for cell synthesis (Lawrence et al. 1978). The two reduced inorganic compounds that have been reported in the literature include reduced iron and sulfur, with responsible bacteria being *Gallionella ferruginea* and *Thiobacillus denitrificans*, respectively (Korom 1992).

Lind (1983) reported following discussions with drinking water managers in Denmark that groundwater containing reduced iron had little or no nitrate. They also found a reverse relationship between nitrate and reduced iron in soil profiles, and concluded that nearly all subsoils were able to reduce nitrate chemically (Lind 1977).

Evidence also has been reported on autotrophic denitrification coupled to reduced sulfur (Frind et al. 1990). The reaction is mediated by the bacteria *Thiobacillus denitrificans* (Kolle et al. 1985, Lawrence et al. 1978). Lawrence et al. (1978) conducted an extensive study on autotrophic denitrification using sulfur of different oxidation states for treating nitrate-containing wastewater. They achieved essentially complete (>99.5%) nitrate removal at steady state. Large amounts of reduced sulfur exist in some aquifers, and the coupling of denitrification to reduced sulfur and sulfate reduction may enhance nitrate removal in groundwater (Korom 1992).

Although some autotrophic denitrification apparently does occur in subsoil and groundwater systems, the more realistic situation may be that it coexists with heterotrophic denitrification. However, organic carbon is preferred thermodynamically as an electron donor whenever it is present in significant quantities (Korom, 1991). Distinction between heterotrophic and autotrophic denitrification is important (Korom 1992), and the roles of these two processes in nitrate removal need to be more fully evaluated.

Denitrification in Subsurface Environments

The potential for groundwater pollution with nitrate derived from fertilizer and waste applications to agricultural soil has stimulated research on denitrification in recent years. For a long time, denitrification has only been studied in surface soil, with its occurrence and significance in the subsurface environment being largely ignored. New evidence has shown that denitrification does occur below the surface soil, however, and may play an important role in determining the eventual fate of nitrate. However, due to changes in chemical, physical and biological characteristics of the soil profile, the contributing factors affecting denitrification in subsurface environment can be greatly different from those in surface soils.

The Subsurface Environment

There is no single definition of subsurface environment and, in fact, this term is often arbitrarily defined and is often dependent upon the context of the discussion. Chapelle (1993b) has classified, based on hydrologic settings, four different subsurface environments: unsaturated soil, water table aquifers, confined aquifers and deep basins. This classification emphasizes the differing degrees of connection with the surface. In reviewing denitrification in the subsurface environment as a potential source for atmospheric nitrous oxide, Rice and Rogers (1993) divided the subsurface environment into two zones. The first zone includes the subsoil and the underlying intermediate vadose zone. This is generally the unsaturated layer between the active root zone and the water table. The second zone is the groundwater or aquifer system. When Pionke and Lowrance (1991) discussed the fate of nitrate in subsurface drainage waters, they divided the soil profile into vadose zone and aquifer. The vadose zone is the soil between the land surface and the groundwater table, which includes the active root zone and the intermediate vadose zone (between root zone and water table). The aquifer can be further divided into unconfined aquifer, semi-confined aquifer and confined aquifer. Despite these differing classifications, it seems that the microbial ecology of subsurface environments has emerged as an important topic due to the fact that subsurface microorganisms play an important role in the mobility and fate of many contaminants (Chapelle 1993a). Certainly denitrification is one of the key processes that determine the fate of nitrate, the most widespread contaminant in groundwater. For the purpose of this review, the subsurface environment is considered to include both vadose zones and shallow and deep aquifers.

Denitrification in Aquifers

There have been some reports on denitrification in deep confined aquifers. Due to the difficulties of direct measurement, most of the denitrification rates were based on indirect estimates such as change in dissolved N_2 and O_2 concentrations (Vogel et al. 1981) and nitrate disappearance. Denitrification rates seem to be variable, though generally low rates are found in deep aquifers.

Compared to deep confined aquifers, shallow unconfined aquifers appear to be more suitable for denitrification. Korom (1992) did an extensive review on denitrification in aquifers and reported both lab and field denitrification rates from many different studies. The denitrification rates varied greatly from site to site. The location, contamination source, aquifer material, sampling depth, temperature, and other measuring conditions apparently contribute to the wide range of rates found in the literature. However, the denitrification rate in a particular environment is ultimately determined by the proximate regulators(or limiting factors) in that environment. The major regulators of denitrification change greatly from site to site.

Trudell et al. (1986) conducted an excellent study on a shallow unconfined sandy aquifer in Canada. They pumped 200 L of groundwater from a depth of 3 meter, which was 2 meters below the water table, spiked it with NO_3^- (as KNO_3) and with the conservative tracer NaBr. The treated groundwater was then injected back into the aquifer. Dilution was monitored by the change in bromide concentration, and any loss of nitrate greater than that which could be accounted for by dilution was attributed to denitrification. Denitrification was also indicated by a decline in dissolved oxygen and an increase in bicarbonate concentration. The denitrification rates were estimated to be from 0.19 to 3.1 mg N L^{-1} d^{-1}.

Starr and Gillham (1989) measured *in situ* denitrification rate in the same sandy aquifer as Trudell et al. (1986), using an acetylene inhibition technique. They pumped out 2L of aquifer material, spiked it with NO_3^- and acetylene, and then monitored N_2O production. The average denitrification rate for a 10 day experiment was 0.58 mg N L^{-1} d^{-1}. The trace amount of NH_4^+-N produced during incubation indicated that dissimilatory nitrate reduction to NH_4^+ was not significant. Starr and Gillham (1989) also conducted a similar study for a deep water table aquifer in an extensive agricultural area. The water table was 4 meters below the soil surface and the groundwater was aerobic, with DO concentration greater than 7 mg L^{-1} in the upper 8 meters of the water table. Nitrate was present at concentrations up to 35 mg N L^{-1} in this zone. They measured no denitrification in this aquifer (<0.014 mg N L^{-1} d^{-1}) despite the presence of denitrifying bacteria. The major difference between these two sites was that in the deep water table site, there was not enough organic carbon to support denitrification, whereas at the shallow water table site the organic carbon was sufficient to sustain high denitrification rates. They concluded that organic carbon availability was an important control on the rate of denitrification in groundwaters. The organic carbon availability also decreased with depth in the subsurface, suggesting that C transformations during transport through the vadose zone were a limiting factor determining the availability of organic carbon in groundwaters.

Carbon limitation of denitrification in an anaerobic groundwater system was also reported by Bradly et al. (1992). In a nitrate contaminated, shallow, sandy aquifer underlying a golf course near Tampa Fl, these authors found an accumulation of nitrate. Based on highly significant correlations between potential denitrification and either sediment total organic content or amended glucose carbon, these authors concluded that denitrification in this aquifer was carbon limited.

However, not all shallow aquifers are carbon limited with respect to denitrification. Slater and Capone (1987) reported that denitrification was limited by nitrate instead of carbon in a nearshore sediment affected by submarine discharge of groundwater. Dilution is another factor affecting nitrate contamination in groundwater or aquifer systems. Howard (1985) reported that denitrification was not significant in the Chalk aquifer of eastern-central England, though it was seriously affected by nitrates leached from agricultural land. Based on major-ion and environmental-isotope data, they concluded that the apparent lowering of nitrate concentrations (from 10 to < 2 mg L^{-1} NO_3-N) in the apparent direction of flow was due primarily to mixing among waters of different origins. This suggests that denitrification can not be solely relied upon to reduce elevated nitrate concentrations in modern recharge waters.

Denitrification in Subsoils and the Intermediate Vadose Zone

Unlike the surface soil and shallow unconfined aquifers, which are both directly related to either agricultural production or environmental problems in associated groundwater resources, denitrification in the subsoil and intermediate vadose zone has not received the same attention by agronomists or environmentalists. However, the microbial processes accruing in this zone play an important role in determining the fate of nitrate in aquifer systems.

Gambrell et al. (1975) studied denitrification in subsoils of the North Carolina coastal plain. The study was conducted in a moderately drained and a poorly drained soil which had 200 kg NO_3-N ha^{-1} and a relatively low level of NO_3-N, respectively. The low Eh, marked decrease of NO_3-N/Cl ratio with depth, and relatively high water-soluble carbon content in the poorly drained subsoil clearly indicated that the low nitrate concentrations in the subsoil were due to enhanced denitrification. The absence of saturated conditions for extended periods of time, and lack of soluble carbon source, apparently limited denitrification in the moderately drained soil.

Denitrification rates generally decrease greatly with depth (Beauchamp et al. 1980, Parkin and Meisinger 1989). The low denitrification rates in subsoils may be the result of many chemical, physical and biological properties which have changed with depth. The most important factor often found to be limiting denitrification in subsoils is the available organic carbon content. Parkin and Meisinger (1989) found that, in a well drained Matapeake silt loam of the Atlantic Coastal Plain, carbon levels below the root zone were too low to support the anaerobic conditions necessary for denitrification or even to sustain a microbial population. Therefore, denitrification was not a mechanism for significant NO_3^--N loss below the root zone.

Many reports have shown that significant denitrification can occur in subsoil environments, with relatively high denitrification rates often being associated with higher organic carbon contents in subsurface soils. Meek et al. (1974) conducted a study examining the effect of large applications of manure on movement of nitrate and carbon in an irrigated desert soil. They found that increased manure application rates increased nitrate levels in the soil solution at 20 and 40 cm, though there was only small differences between 80 and 140 cm. They attributed the lack of nitrate of the greater depths to increased denitrification due to organic carbon leached to this depth from the surface. Increased denitrification in subsoils and groundwaters following addition of organic carbon to the subsoil were also reported by Comfort et al. (1990), Obenhuber and Lowrance (1991), and Weier et al. (1994).

Another important factor that can create conditions conductive for denitrification in the subsoil is the change of morphological properties such as texture and bulk density. Gilliam et al. (1978) observed a general relationship between soil texture and denitrification for four California soils. They concluded that the relation between denitrification and texture was actually the result of impeded water flow, which created conditions conductive to denitrification. Devitt et al. (1976) also observed that subsoil denitrification was possible in coarse-textured soils that contained a subsurface layer of higher clay content.

McGarity and Myers (1969) measured denitrification activity in 17 soil profiles from eastern Australia, where heavy-textured B horizons with low permeability were found. They observed that denitrification activity was stratified and variable within the soil profile, with increased denitrification activity being associated with the upper portion of the B_{2t} horizon. They later concluded that significant subsoil denitrification activity was due to transitory accumulations of organic carbon in the subsoil following leaching of highly available soluble organic carbon from the surface soils (Myers and McGarity 1971).

Denitrification in Riparian Subsoils

It becomes increasingly clear that riparian ecosystems can be crucial in maintaining the quality of streamflow following both subsurface and surface discharge from agricultural soils. High nitrate concentrations have been found in shallow groundwater from the coastal plain of Georgia, North Carolina and Maryland (Gilliam et al. 1974, Lowrance et al. 1984, Peterjohn and Correll 1984). These shallow, unconfined aquifers, supplying as much as 80% of the total streamflow in coastal plain streams (Shirmohammadi et al. 1986) are heavily impacted by nitrate from agricultural watersheds. However, elevated nitrate concentrations have not been found in the streams originating from these watersheds. The riparian systems located between the agricultural lands and the streams may be largely responsible for the low nitrate levels in discharge waters (Gambrell et al. 1975, Jacobs and Gilliam 1985, Lowrance et al. 1985). Denitrification is assumed to be the primary mechanism for nitrate removal in these riparian ecosystems. The high organic carbon content and anaerobic conditions in the riparian subsoil provide an ideal environment for denitrification, which can quite effectively remove most of the nitrate that is being transported through the riparian zones.

There have been a number of recent studies on denitrification in riparian zones of New Zealand and Europe (Ambus and Christensen 1993, Schipper et al. 1989, Schipper et al. 1991, Schipper et al. 1993). In a very narrow (< 5m) streamside riparian zone, Schipper et al. (1993) found that most nitrate entering into this riparian zone with groundwater had been removed before reaching the stream. They observed a denitrification rate of 1.12 g N m^{-2} day^{-1}, which was 1-3 orders of magnitude greater than reported for upland soils. The high organic carbon content, anaerobic conditions and abundant supply of nitrate from the incoming groundwater resulted in an ideal environment for denitrification at this site.

Although many researchers have demonstrated that riparian ecosystems could sustain a considerable denitrification activity, both the magnitude of denitrification and major regulators vary greatly among systems. Since the environmental conditions in most riparian zones are conductive for denitrification, denitrification is generally regulated (limited) by the factor which is present in lowest supply, quite often the NO_3^--N concentration (Schipper et al. 1993).

Conclusions

Denitrification has been studied for more than 100 years, and considerable amounts of information have been generated regarding the diversity and physiology of microbes responsible for denitrification and effects of environmental factors on the denitrification process. However, the effects of environmental factors such as oxygen, nitrate concentration, organic carbon, temperature and pH on the establishment and maintenance of denitrification at a landscape scale is not yet fully understood. The complex soil environment creates a continuum of levels for each of the factors; therefore, partial inhibition may occur in a system. Denitrification is rarely fully expressed in a natural system and is often limited by one or two factors in a given environment.

Research in the past has been centered on investigating and minimizing denitrification in agricultural surface soils due to the concern over N fertilizer loss. Because of the increasing evidence of groundwater nitrate contamination, however, more and more studies have been reported on denitrification processes in subsurface environments, especially nitrate-contaminated groundwater and aquifer systems. The rates and regulators of denitrification in subsurface environment are far from fully understood.

The following are some of the areas that need further study:

(1) *In situ* denitrification rates in subsoils and groundwater. The focus here should be on obtaining representative soil samples and determining the oxygen status of the subsoil environment so that denitrification measurements can be done under conditions similar to ambient soil conditions.

(2) Regulators on denitrification in subsoils. Organic carbon plays an important role in both surface and subsurface soils. Transport of dissolved organic carbon in the soil column and its bioavailability to denitrifiers in subsoils need to be further studied. Topics in this area may include the rate of DOC transport in soil columns, and organic-C decomposition in subsoils. One of the major difficulties in studying the bioavailability of organic-C to denitrifiers is lack of a reliable method for quantifying available C levels.

(3) Carbon sources for denitrification in subsoils. A promising study area is to investigate possible C sources for enhancing denitrification in nitrate-contaminated groundwater systems.

CHAPTER 3

EFFECT OF DAIRY EFFLUENT IRRIGATION ON GROUNDWATER QUALITY AND SOIL CHEMISTRY

Introduction

Nitrate is the most widespread chemical contaminant in groundwater throughout the world (Spalding and Exner 1993). Groundwater nitrate concentrations have been increasing over the last few decades in Europe (Spalding and Exner 1993) as well as in the USA (Hallberg 1989). Excessive intake of nitrate can cause methemoglobinemia, also known as "blue baby syndrome", cancer and possibly other health-related problems (Keeny 1989). High NO_3^- levels in groundwater can also affect surface water resources such as streams and rivers when the nitrate contaminated groundwater discharges to these surface water bodies (Hallberg 1989, Reay et al. 1992, Spalding and Exner 1993,).

With respect to pollution sources, it is generally agreed that intensive agricultural land use contributes significantly to increased nitrate concentrations in groundwater (Follett and Walker 1989, Keeny 1989). Over the past ten years, annual N fertilizer consumption has increased nearly 25% in developed countries and nearly 160% in developing countries (Follett and Walker 1989). Many researchers have found a direct relationship between nitrate levels in groundwater and the amount of N fertilizer used in agriculture (Hallberg 1989).

Animal manures are another source of nitrate for groundwater. The nitrogen (N) in such waste materials is often in organic form. Nitrogen transformations after application to the soil make the N slowly available to plants. Therefore, N in the effluent is not generally subject to rapid leaching compared to that from inorganic N fertilizer. However, extensive use of organic wastes such as animal manures may also cause nitrate accumulation in groundwater, especially for light textured soils.

Although nitrate pollution in shallow groundwater has been studied in the coastal plain of Georgia (Harbbard et al. 1984, Lowrance 1992, Lowrance et al. 1984), North Carolina (Gilliam et al. 1974, Gilliam et al. 1979, Jacobs and Gilliam 1985), and Maryland (Jordan et al. 1993, Weil et al. 1990), there is little such information available for Florida.

Application of dairy lagoon effluent through irrigation system is a common practice for dairy farms in north and north central Florida. The number of dairy farms in Florida, especially in north Florida, has increased greatly over the last 20 years (Andrews 1992). This has raised concern over nitrate contamination in the Upper Floridan Aquifer of the Floridan Aquifer system, which is the major portable water source for north Florida. The upper portion of the aquifer is unconfined and generally underlain with very thin, highly permeable sandy soil. Therefore, it is vulnerable to nitrate contamination.

Concerns also arise because the shallow aquifers of the southeastern US supply as much as 80% of the total stream flow for the coastal plain streams (Shirmohammadi et al 1986). Therefore, nitrate pollution in these aquifers may have adverse effects on such surface water resources.

In this study, we investigated the impact of dairy lagoon effluent irrigation on water quality in a unconfined shallow aquifer. Our purpose was to determine the extent of nitrate pollution in this aquifer and its relation to groundwater hydrology and soil properties.

Materials and Methods

Study Site

The study was conducted at the Dairy Research Unit (DRU) of the University of Florida, about 19 km north of Gainesville, FL. The site consisted of a cropped field and adjacent riparian forest (Fig. 3-1). Corn, sorghum and rye grass were rotated in the field to provide either animal feed for the dairy farm or green cover. The field had been irrigated with dairy lagoon effluent using a center-pivot irrigation system for 5 years. Irrigation was carried out once or twice each week between March and October,

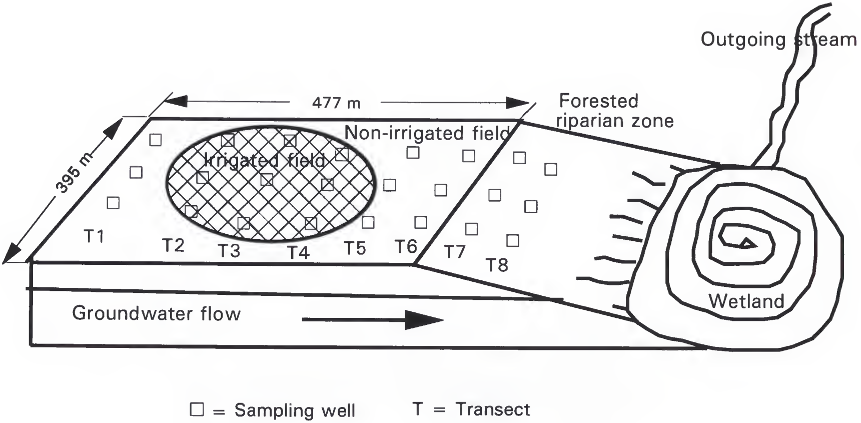

Fig. 3-1. Schematic diagram of the study site.

when corn and sorghum were growing. Irrigation was less frequent during the winter. The amount of water applied each time varied from 0.5 to 3 cm, with an average of approximately 1 cm. No N-fertilizer was applied to the effluent irrigated field. However, N-fertilizer was applied on March 14, 1994 to the non-irrigated cropped areas at the rate of 190 kg N per hectare. The riparian zone was located between the crop field and a wetland swamp in the downgradient forest. Native vegetation in the riparian forest included hardwood trees such as slash and longleaf pine, and water and live oak trees. The understory consisted of waxmyrtle, sumac, blackberry, gallberry, and scattered salpalmetto, along with carpetgrass and other native weeds and grasses.

Soils in the irrigated field are primarily Tavares Sand and Chiply Sand (thermic coated Aquic Quartzipsamments) and the soil in the riparian forest was Chiply Sand (thermic coated Aquic Quartzipsamments) (Thomas et al. 1985). Although the soils were classified from moderately well drained (Tavares Sand) to somewhat poorly drained (Chiply Sand), the groundwater table fluctuated between <20 and 200 cm during the year. Irrigation brought the water table even closer to the soil surface during the summer rainy season. Due to the sharp change in surface elevation from the cropped field to the wetland, groundwater drained from the field, through the riparian zone, towards the wetland.

Groundwater seepage reached the soil surface in the lower portion of the riparian slope.

Groundwater Chemistry

Soil surface elevation survey

The soil surface elevation was surveyed in the study area prior to well installation. The survey was conducted by selecting the highest (southeast) corner of the area as the reference point. However, the lowest water table depth during the study period (on June 1994) in the riparian zone was subsequently used as the reference point during graphing, to avoid dealing with negative values.

Well installation

A series of wells were installed along the hydraulic gradient in the study site on February 17, 1994 (Fig.3-1). The study area was divided into five zones based on location within the hydrologic gradient and anticipated degree of impact by nitrate. The five zones were: up-gradient non-irrigated zone (UPGRAD), irrigated zone (IRRI), down-gradient non-irrigated zone (DOWNGRAD), edge of the field (EDGE), and riparian zone (RIPARIAN). The first transect in the riparian zone was called RIPARIAN 1, the second RIPARIAN 2. The irrigated zone was located in the center of the field, occupying about 50% of the crop area. The wells were arranged in such a pattern that every zone was represented.

However, the number of wells in each zone varied according to size of the area. A total of 8 transects were set up, with three wells in each transect (Fig. 3-1).

Two wells were installed at each well location with one being shallow and one being deep. The shallow well consisted of a 91 cm (length) x 3.8 cm i.d. solid PVC pipe with 30 cm of well screen near the bottom. The deep well was 152 cm long instead. The wells were placed into the ground with only about 10 cm left above the soil surface. The two wells were located about 1 meter apart, to minimize interferences with each other.

Groundwater sampling

Groundwater samples were taken approximately monthly between February 26, 1994 and December 1, 1994. Water table depth was measured on each sampling date before the water sampling began. The wells were then emptied completely, twice, using a portable vacuum pump. When the well had again refilled with fresh water, about 100 ml of water sample was taken and immediately filtered through a 0.45 μm filter using a syringe-type filtering device. The water samples were returned to the lab on ice and stored at 4^{0}C until analyses were completed. If the samples could not be analyzed within two days, they were frozen at -4 ^{0}C.

After the water samples had been taken, the dissolved oxygen (DO) content of the refreshed well water was determined using a Yellow Springs Instrument DO meter with a YSI DO probe gently lowered into the well. The meter was calibrated against water-vapor-saturated air before the reading was taken. Water temperature was also measured, using the built-in thermometer in the DO probe.

Chemical analyses

The pH of the water samples was taken within 24 hours of sampling using an Orion pH meter. The samples then were analyzed for NO_3^--N using an automated cadmium reduction method (method 418 C, APHA 1989), and for NH_4^+-N using an automated salicylate method (method 361.2, EPA 1983), respectively. Check standards (EPA) were used to confirm the methods, with recovery being within the accepted 95% confidence limits. Sample $SO_4^=$ content was analyzed using a Dionex 4500i Ion Chromatograph equipped with a conductance detector and anion micro-membrane suppressor (AMMS-1). Separation from other anions was accomplished using an Ionpac AS4A analytical column and an Ionpac AG4A guard column with 1.8 mM Na_2CO_3/1.7 mM $NaHCO_3$ as the eluant. The Cl^- ion was analyzed using the same method, though with 5 mM tetraborate eluant solution. Dissolved carbon (DC), dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC) were analyzed using a high temperature Dohrmann DC-190 TOC Analyzer. The carbon analyses were also checked against EPA standard solution.

Groundwater flow

The rate of groundwater flow was calculated using Darcy's Law. Darcy's Law states that flow rate through a porous medium is proportional to the hydraulic head difference, inversely proportional to the length of the flow path, and proportional to the hydraulic conductivity of the porous medium. This law is valid as long as laminar flow is maintained (Todd 1980).

The mathematical form of Darcy's law can be stated as:

$$q = -K(\Delta H/\Delta X)$$

where

q = Darcy's velocity ($L\ T^{-1}$)
K = hydraulic conductivity of the soil ($L\ T^{-1}$)
ΔH = the hydraulic head loss (L)
ΔX = distance (L)

Soil Chemistry

Soil sampling

Intact soil cores were taken four times between June and December 1994, to a depth of 120 cm using an Environmentalists Subsoil Probe (Clements Assoc. Inc.). Three soil cores were taken in each zone, approximately 5 meters from the sampling wells. The intact soil cores then were transported back to the lab where they were sectioned at 20 cm intervals. Due to the wetness of the soil, compaction was measured at 20 cm intervals and taken into consideration during the sectioning. For each 20 cm equivalent, the depth between the top of the sampling probe and the soil surface both inside and outside the probe were measured, with the difference between these two depths reflecting the degree of compaction for that interval depth. For example, if the difference for the first 20 cm soil core was 2 cm, the compaction was 2 cm and only 18 cm of soil core was cut for the first 20 cm section. The soil samples were stored in plastic sample bags at 4°C.

Soil extraction and analyses

Moisture contents of the soil samples were determined by oven drying approximately 20 grams of wet soil at 105°C for 24 hours. For water extraction of nutrients, approximately 10 grams of moist soil were weighed into 50 ml centrifuge tubes to which 20 ml of distilled deionized water (DDI) were added. After shaking for 1 hour, the soil suspensions were centrifuged at 8,000 rpm for 15 minutes and then filtered through Gelman 0.45 μm membrane filters. The solutions were stored at 4°C until analyses. The water extracts were analyzed for NO_3^--N, NH_4^+-N, $SO_4^=$-S, Cl^-, DC, DIC, and DOC in the same way as for the groundwater samples.

Finely ground air-dried soil samples from August 1994 were analyzed for total nitrogen and carbon using a Carlo-Erba NA-1500 CNS analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). The pH values of August samples were determined in 1:2 soil:water suspensions using an Orion pH meter.

Dairy effluent from both primary lagoon and the retention pond were sampled and characterized for NO_3^--N, NH_4^+-N, pH, TKN, dissolved carbon and dissolved organic carbon using the same methods as described earlier.

Data Analysis

Statistical analysis was done using SAS version 6.09 (SAS Institute Inc. 1992). Analysis of variance was carried out using PROC GLM procedure based on a split-plot design. The Least Squares Means procedure was used to compare differences among the water quality and soil chemical parameters. All differences are reported as significant in a rejection region of $p \leq 0.05$.

Results

Groundwater Hydrology

Soil topography

The soil surface elevation decreased in both the western and northern directions (Fig. 3-2). The study area

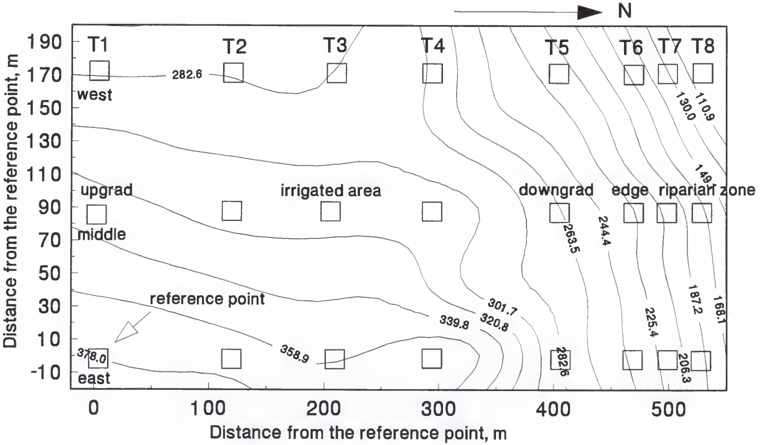

Fig. 3-2. Topographic map of the study site. The units of the contour lines are in centimeters (cm). Each square represents a sampling well. T = transect consisting of three wells in east-west direction. T1 = upgradient, T2, T3, T4= irrigated, T5 = downgradient, T6 = edge, T7 = riparian 1, T8 = riparian 2.

can be divided into two distinct subareas according to topography (Fig. 3-3). A generally flat area covered the upgradient zone and most of the irrigated field, with a slope of only 0.4% in the east-west direction and little change in the north-south direction. A relatively steeper slope was found between the irrigated perimeter of the field and the riparian zone, with a slope of 1.1% in the south north direction.

Groundwater table depth and flow direction

The groundwater table depth generally followed the same pattern as the soil surface elevation. For illustration, the groundwater elevation pattern for February is shown (Fig. 3-4). Groundwater table depths evidenced little variation in the upgradient zone and the irrigated field. However, the water table depths dropped consistently between the irrigated field and the riparian zone (Fig. 3-5). Therefore, the water table changes along the south-north direction also formed two distinct sections. These are designated as "plateau", from the upgradient zone to the last transect of wells in the irrigated zone, and "slope", which covered from the edge of the irrigated circle to the last transect of wells in the riparian zone, respectively (Fig. 3-5).

The water table depth also decreased in the east-west direction; however, the change was much less than that in the north-south direction (Fig. 3-5). Based on the water

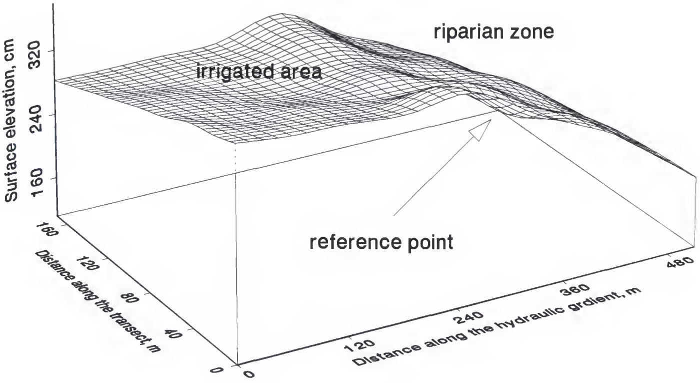

Fig. 3-3. Soil surface elevations relative to the lowest water table depth.

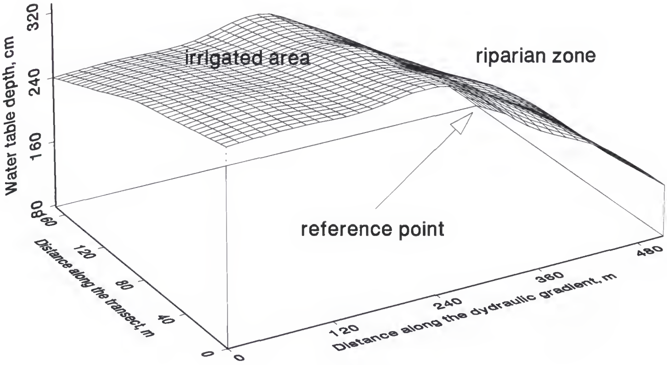

Fig. 3-4. Groundwater table altitudes relative to the lowest water table depth in the study area. (Data shown are from February 1994).

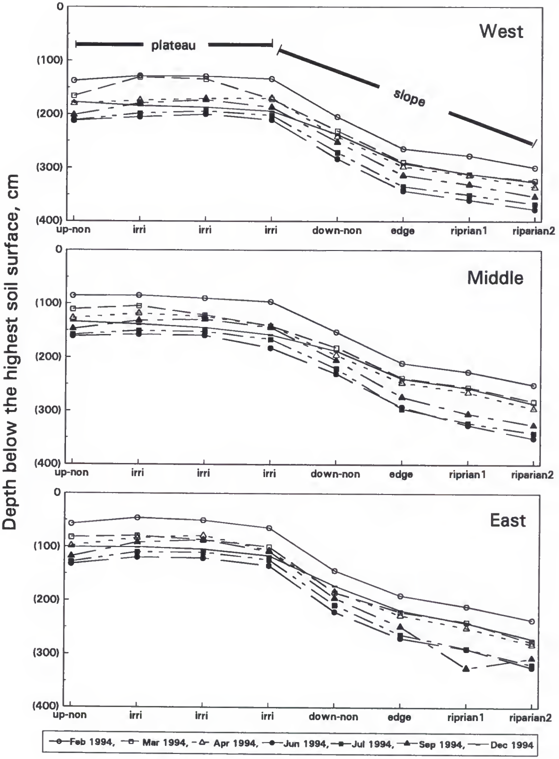

Fig. 3-5. Groundwater table depth along the hydraulic gradient for different sampling dates.

table elevations, the groundwater in the study area can be reasonably assumed to flow from the irrigated field towards the northwest corner of the riparian zone.

Groundwater table depths also showed seasonal variations (Fig. 3-5). The highest water table occurred in February 1994, and the lowest was in June 1994. The range in water table fluctuation was about 90 cm during the study period, and the pattern between the plateau and slope areas remained relatively constant.

Groundwater flow rate

An estimate of the soil hydraulic conductivity was obtained from the soil survey of Alachua County (Thomas et al. 1985). Tavares Sand and Chipley Sand occupied the irrigated field (plateau) and Chipley Sand was located between the irrigated field and the riparian zone (slope). The hydraulic conductivities at the depth of 60-120 cm would be approximately 40 and 26 cm hr^{-1} for the "plateau" and "slope" areas respectively.

Values for the hydraulic head loss in the "plateau" and "slope" areas were calculated based on the change in water table depth for these two areas. The groundwater flow rate (m yr^{-1}) was calculated using Darcy's Law (Table 3-1).

Groundwater movement in the "plateau" area was very slow, with an estimated flow rate of only 0.1 m yr^{-1}. The estimated flow rate in the "slope" area was 15.9 m yr^{-1}.

Table 3-1. Groundwater flow rates in the plateau and slope areas in the north-south direction†

Location	K	ΔH	ΔX	Flow rate
	cm hr^{-1}	m	m	m yr^{-1}
Plateau	40	0.0062	277	0.1
Slope	26	1.65	235	15.9

† K is the hydraulic conductivity, ΔH is change in the hydraulic head, and ΔX is distance along the hydraulic gradient.

Groundwater Chemistry

The examined groundwater parameters included NO_3^--N, NH_4^+-N, NO_3:Cl ratio, $SO_4^=$-S, TDC, TDOC, pH, temperature, and dissolved oxygen. Change in these parameters along the hydraulic gradient and with time in the study area are presented in the following sections. There was no water in the shallow wells during June, July and September of 1994.

Nitrate-N

The upgradient zone groundwater (control) had average NO_3^--N concentrations of less than 2 mg L^{-1} and showed no seasonal variation during the study period (Table 3-2). Nitrate-N concentrations in irrigated-zone groundwater were significantly higher than those in the upgradient, field edge and riparian zones and did not show appreciable change with time (Table 3-2). High NO_3^--N concentrations were also found in the shallow wells of the downgradient zone; however, these high NO_3^--N concentrations occurred only in March and April. The shallow wells in the field edge and riparian zones had little NO_3^--N and no marked seasonal variations.

Elevated NO_3^--N concentrations were also found in deep wells of the irrigated and downgradient zones, with concentrations over 10 mg L^{-1} for many of the sampling dates (Table 3-3). The peak concentration of NO_3^--N in deep wells of these two zones appeared in March and April and generally

Table 3-2. Change of NO_3^--N concentrations in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	--------------------mg L^{-1}--------------------			
upgrad	3.78aA‡	2.10aA	1.02aA	0.97aA
irrigated	17.28bA	22.97bA	18.36bA	18.01bA
downgrad	3.27aA	21.45bB	26.11bB	2.61aA
edge	1.04aA	0.72aA	2.56aA	5.38aA
riparian1	1.10aA	1.70aA	0.16aA	1.46aA
riparian2	1.06aA	0.05aA	0.05aA	3.26aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-3. Change of NO_3^--N concentrations in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
	mg L^{-1}						
upgrad	1.52aA‡	2.36aA	3.21aA	2.81aA	2.73aA	1.56aA	6.18abA
irrigated	15.23bBCD	21.57bE	19.84bDE	14.56bBC	18.05bCE	10.80bAB	9.42bA
downgrad	9.36abABCD	17.85bDEF	21.74bEF	12.98bBDE	18.73bE	7.83abAB	4.05abA
edge	1.87aA	0.09aA	1.19aA	0.97aA	2.28aA	0.89aA	5.12abA
riparian1	1.29aA	0.93aA	1.81aA	1.12aA	1.51aA	0.48aA	1.67aA
riparian2	1.03aA	0.00aA	0.07aA	0.01aA	0.02aA	0.34aA	7.70abA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

decreased thereafter. Deep wells in the upgradient, field-dedge and riparian zones had low NO_3^--N concentrations and little seasonal variation.

Although groundwater samples from the shallow wells sometimes showed higher NO_3^--N concentrations, the overall concentration (average over all dates) in the shallow wells was not significantly higher than that in the deep wells (Fig.3-6).

Ammonium-N

Ammonium-N concentrations in groundwater of both the shallow and deep wells were generally low, with concentrations being less than 1 mg L^{-1} (Tables 3-4, 3-5). Irrigation with effluent appeared to have no effect on NH_4^+-N levels in the groundwater, though NH_4^+-N concentrations in both the shallow and deep wells showed significant increases in the riparian zone several times during the study period. The upgradient, irrigated, downgradient and field edge zones had low NH_4^+-N concentrations and showed almost no seasonal variation.

There was no significant difference between the shallow and deep wells in all zones except for the first transect in the riparian zone, where the shallow wells had higher NH_4^+-N concentrations than did the deep wells (Fig. 3-7).

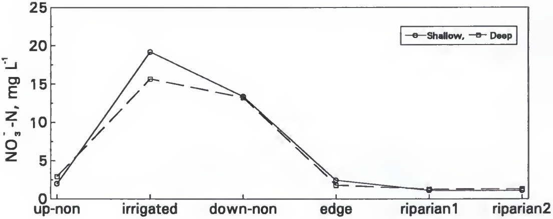

Fig. 3-6. Averaged groundwater NO_3^--N concentrations in shallow and deep wells along the hydraulic gradient. There was no significant difference between the two depths.

Table 3-4. Change of NH_4^+-N concentrations in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	------------------mg L^{-1}-------------------			
upgrad	0.045aA‡	0.035aA	0.032aA	0.120aA
irrigated	0.041aA	0.055aA	0.064aA	0.071aA
downgrad	0.074aA	0.042aA	0.115aA	0.040aA
edge	0.088aA	0.032aA	0.042aA	0.070aA
riparian1	0.048aA	0.036aA	0.549bB	0.306aA
riparian2	0.128aA	0.572bB	0.110aA	0.115aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-5. Change of NH_4^+-N concentrations in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
	mg L^{-1}						
upgrad	0.046abA‡	0.032aA	0.031aA	0.030aA	0.024aA	0.165aA	0.207aA
irrigated	0.051aA	0.048aA	0.049aA	0.195aA	0.037aA	0.224aA	0.038aA
downgrad	0.039abA	0.053aA	0.034aA	0.030aA	0.024aA	0.196aA	0.038aA
edge	0.047abA	0.050aA	0.044aA	0.143aA	0.052aA	0.757bB	0.079aA
riparian1	0.301abA	0.124aA	0.065aA	0.178aA	0.026aA	0.150aA	0.106aA
riparian2	0.349bAB	0.072aA	0.112aA	0.293aAB	0.550bB	0.292aAB	0.205aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

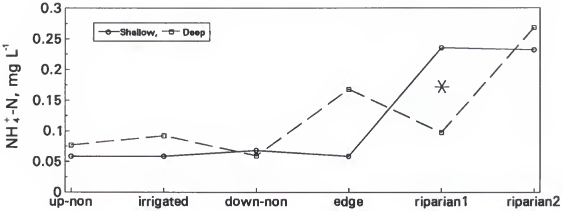

Fig. 3-7. Averaged groundwater NH_4^+-N concentrations in shallow and deep wells along the hydraulic gradient. The asterisk designates a significant difference between the two depths.

Sulfate-S

Sulfate-S concentrations ranged from 5.1 to 14.4 mg L^{-1} and showed no particular trend along the hydraulic gradient in either the shallow or deep wells (Tables. 3-6, 3-7). Sulfate-S concentrations of the shallow and deep wells in most zones also showed little seasonal variation. The zones showing seasonal variations were the irrigated zone (in both shallow and deep wells) and the riparian 1 zone (in deep wells). However, no particular pattern of seasonal variation was found in these two zones. When averaged over time, only the downgradient and edge zones had different $SO_4^{=}$-S concentrations at the two depths (Fig. 3-8). The deep wells in the downgradient zone had higher $SO_4^{=}$-S concentrations, but the opposite was found in the edge zone.

Nitrate to chloride ratio (NO_3:Cl)

Change of NO_3:Cl ratios along the hydraulic gradient in both shallow and deep wells followed the same pattern as groundwater NO_3^--N concentrations (Tables 3-8, 3-9). Increased NO_3:Cl ratios occurred in irrigated and downgradient-zone groundwater. The ratios decreased as groundwater left the irrigated zone and flowed through the riparian zone.

Shallow wells in the upgradient, field edge, riparian 1 and 2 zones showed no seasonal variations in NO_3:Cl ratios (Table 3-8), and the same was true for the deep wells except

Table 3-6. Change of $SO_4^=$-S concentrations in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	-------------------	-mg L^{-1}	-------------------	
upgrad	6.0aA‡	6.8aA	7.4abA	6.9abA
irrigated	8.9bA	9.2bA	11.6cB	11.0cB
downgrad	8.2bA	7.5abA	7.2abA	6.8abA
edge	11.7cA	11.5cA	10.2bcA	9.9bcA
riparian1	10.2cbA	12.6cA	9.8bcA	9.6bcA
riparian2	7.6abA	6.8abA	5.6aA	5.5aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-7. Change of $SO_4^{=}$-S concentrations in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
	---------------------------	-------------------	-------------------	mg L^{-1}	-------------------	-------------------	-------------------
upgrad	7.6aA‡	7.0aA	7.3abA	7.4acA	7.4abA	5.9aA	6.7abA
irrigated	9.5aA	10.4bcA	9.8bA	13.4bB	14.4cB	14.3cB	9.7bA
downgrad	9.6aA	8.7abA	9.4bA	11.2abA	11.4bcA	10.1bA	8.8abA
edge	8.3aA	8.6abA	8.7abA	8.3acA	8.6abA	8.7acA	8.0abA
riparian1	10.4aAB	13.3cB	10.4bAB	10.5abAB	10.7bAB	9.1acA	10.3bAB
riparian2	7.1aA	7.4abA	5.1aA	6.5cA	5.8aA	5.4aA	5.9aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

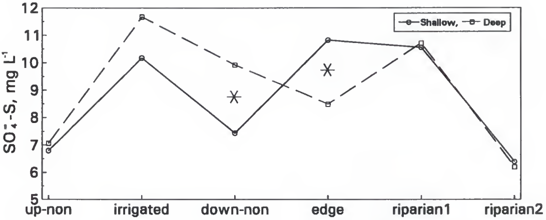

Fig. 3-8. Averaged groundwater $SO_4^{=}$-S concentrations in shallow and deep wells along the hydraulic gradient. The asterisks designate significant differences between the two depths.

Table 3-8. Change of NO_3:Cl ratios in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	mg L^{-1}			
upgrad	0.18bcA‡	0.11aA	0.05aA	0.07aA
irrigated	0.48cA	0.72bAB	0.59bAB	0.78bB
downgrad	0.11bcA	0.73bB	0.79bB	0.09aA
edge	0.08aA	0.09aA	0.14aA	0.44aA
riparian1	0.03aA	0.06aA	0.01aA	0.08aA
riparian2	0.04aA	0.00aA	0.00aA	0.12aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-9. Change of NO_3:Cl ratios in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
				mg L^{-1}			
upgrad	0.09aA‡	0.08aA	0.29aA	0.08abcA	0.05aA	0.05aA	0.62cB
irrigated	0.37bA	0.59bB	0.65bB	0.29cA	0.38bA	0.24aA	0.34bA
downgrad	0.34abA	0.71bB	0.72bB	0.32bcA	0.37bA	0.17aA	0.14abA
edge	0.06aA	0.01aA	0.03aA	0.03abA	0.06aA	0.02aA	0.27abA
riparian1	0.06aA	0.06aA	0.09aA	0.04abA	0.03aA	0.01aA	0.07aA
riparian2	0.03aAB	0.00aA	0.01aA	0.00aA	0.00aA	0.01aA	0.33abcB

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

for December, where the ratios increased in the upgradient and riparian 2 zones. Although the ratios were high for the shallow wells in the irrigated zone, the seasonal change was small (Table 3-8). Shallow wells in the downgradient zone had high NO_3:Cl ratios in March and April of 1994 and low NO_3:Cl ratios in February and December of 1994.

Deep wells in the irrigated and downgradient zones had a period of high NO_3:Cl ratios during March and April of 1994 (Fig. 3-9), and then decreased after April. The deep wells in the other zones had relatively constant NO_3:Cl ratios from February to September of 1994. Nitrate to chloride ratios in deep wells of the upgradient and riparian 2 zones in December were higher than those in previous months.

Average NO_3:Cl ratios over all sampling dates showed that only the irrigated zone shallow wells had higher NO_3:Cl ratios than did deep wells (Fig. 3-9). The ratios in all other zones did not show a significant difference between the shallow and deep wells.

Dissolved carbon (DC)

Dissolved species are defined herein as chemical constituents that can pass through a 0.45 μm filter. Dissolved carbon (DC) consisted of dissolved inorganic carbon (DIC) and dissolved organic carbon (DOC).

The shallow wells of the downgradient, edge and

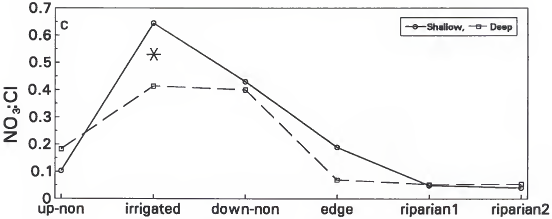

Fig. 3-9. Averaged groundwater NO_3:Cl ratios in shallow and deep wells along the hydraulic gradient. The asterisk designates a significant difference between the two depths.

riparian 1 zones had relatively constant DC concentrations and little seasonal variation.

The shallow wells in the irrigated zone showed higher DC concentrations than other zones on almost all sampling dates (Table 3-10). High DC concentrations were also observed in riparian 2 zone shallow wells during February and December of 1994.

Dissolved carbon concentrations in deep wells of the irrigated zone were also higher than those of other zones on all sampling dates except December 1994 (Table 3-11). However, the higher DC concentrations found in shallow wells of the riparian zone were not observed in the deep wells. Lowest DC concentrations were observed in both the shallow and deep wells in December 1994 (Tables 3-10, 3-11). Only the irrigated zone and the last transect of the riparian zone had significantly higher DC concentrations in shallow wells than in deep wells (Fig. 3-10).

Dissolved organic carbon (DOC)

Dissolved organic carbon (DOC) concentrations in both the shallow and deep wells were appreciably lower than DC concentrations. Shallow wells in the irrigated zone showed higher DOC concentrations than in the upgradient, downgradient, field edge and riparian 1 zones (Table 3-12). Shallow wells in the riparian 2 zone also showed higher DOC concentrations for all dates except March (Table 3-12). The

Table 3-10. Change of dissolved carbon (DC) concentrations in shallow wells along the hydraulic gradient on different sampling date (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	mg L^{-1}			
upgrad	19.4aB‡	19.8aB	15.1aAB	4.5aA
irrigated	36.4bC	34.4bC	26.8bB	14.7bA
downgrad	17.3aA	13.5aA	9.3aA	7.0abA
edge	15.9aA	15.3aA	10.0aA	7.2abA
riparian1	14.8aA	13.2aA	12.8aA	5.4aA
riparian2	36.9bB	17.3aA	16.8aA	13.3abA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-11. Change of dissolved carbon (DC) concentrations in deep wells along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
				mg L^{-1}			
upgrad	18.7bD‡	16.6aCD	12.4aC	20.1bD	13.0aC	25.9bB	6.9aA
irrigated	25.2cCD	23.3bC	17.1bB	26.0dCD	18.8bB	26.9bD	8.4aA
downgrad	17.2abC	14.9aBC	11.6aB	17.4bcC	10.4aB	14.1aBC	6.4aA
edge	15.4abC	15.2aC	10.8aBC	12.3acBC	8.4aAB	13.1aBC	5.6aA
riparian1	13.0aB	12.2aB	9.6aAB	12.5acB	8.5aAB	11.4aB	4.5aA
riparian2	15.2abB	13.5aAB	9.6aA	11.4aAB	8.7aA	11.0aAB	9.3aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

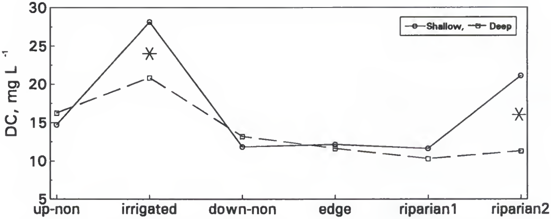

Fig. 3-10. Averaged groundwater dissolved carbon (DC) concentrations in shallow and deep wells along the hydraulic gradient. The asterisks designate significant differences between the two depths.

Table 3-12. Change of dissolved organic carbon (DOC) concentrations in shallow wells along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	----------	---------- mg L^{-1}	----------	----------
upgrad	11.4aB‡	12.1aB	8.4abB	3.2aA
irrigated	17.6bB	17.2bB	10.6bA	8.7bcA
downgrad	11.8aC	9.4aCB	6.2aBA	5.2aA
edge	11.3aB	11.6aB	6.6aA	5.8abA
riparian1	12.5aC	9.6aCB	7.8abAB	4.6aA
riparian2	19.2bB	12.4aA	11.5bA	10.2cA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

DOC concentrations in shallow wells of both the irrigated and riparian-zone areas were high during February and March and decreased in April, with the lowest concentrations observed in December 1994.

Unlike the shallow wells where the irrigated zone had higher DOC concentrations on all sampling dates, higher DOC concentrations were only observed in the irrigated zone in February, March, June and September (Table 3-13). Higher DOC concentrations in the deep wells of the riparian zone were only observed in February 1994.

Annual average DOC concentrations were higher in shallow wells than those in deep wells in the irrigated and riparian 2 zones (Fig. 3-11). Other zones showed similar levels of DOC at both depths.

Dissolved oxygen (DO)

Dissolved oxygen measurements were carried out from April to December of 1994. Dissolved oxygen concentrations in shallow wells were generally less than 2 mg L^{-1} within the field area (Table 3-14). However, shallow wells in the riparian zone had unusually high DO concentrations in April 1994, probably because the wells were almost dry at the sampling time, so that only the oxygenated surface layer of the groundwater could enter the wells. Dissolved oxygen concentrations in the shallow wells did not show any difference between April and December except for the two

Table 3-13. Change of dissolved organic carbon (DOC) concentrations in deep wells along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
	mg L^{-1}						
upgrad	11.2abC‡	10.3abC	6.7aAB	9.2abBC	6.0aA	9.6abC	4.5aA
irrigated	13.9cD	13.4cD	7.6aB	10.4bC	7.1aB	9.9bC	4.8aA
downgrad	11.1abE	9.5abCDE	6.4aB	8.3abBCE	5.7aB	8.5abC	4.2aA
edge	11.4abD	10.8abCD	6.2aAB	8.4abBC	6.1aAB	7.1aB	4.4aA
riparian1	9.4aD	8.2aCD	6.3aABC	8.2aCD	5.0aAB	7.4aBCD	3.7aA
riparian2	12.1bcC	11.3bC	6.7aAB	8.4abB	5.5aA	7.2aAB	7.2bAB

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

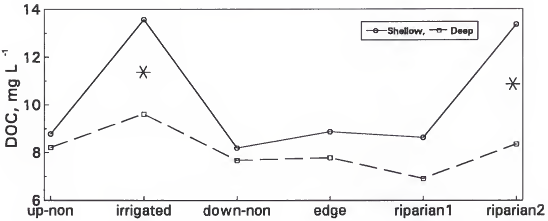

Fig. 3-11. Averaged groundwater dissolved organic carbon (DOC) concentrations in shallow and deep wells along the hydraulic gradient. The asterisks designate significant differences between the two depths.

Table 3-14. Change of dissolved oxygen (DO) concentrations in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Apr.	Dec.
	------------mg L^{-1}	------------
upgrad	1.3aA‡	1.1aA
irrigated	1.3aA	1.6aA
downgrad	1.9abA	2.0aA
edge	1.9abA	1.3aA
riparian1	6.4bB	2.1aA
riparian2	4.2bB	1.3aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

higher DO values in the riparian zone (Table 3-14).

Dissolved oxygen concentrations in deep wells were also low, with an upper level of 1.3 mg L^{-1}. Average DO concentrations over time were less than 1 mg L^{-1} in deep wells of all zones. Although there were some spatial and seasonal variations in DO concentrations, the differences were small (Table 3-15).

The shallow wells had relatively higher DO concentrations in general than did the deep wells (Fig. 3-12). Significantly higher DO concentrations were only observed in shallow wells of the riparian zone, apparently due to measurements from the nearly dry wells. Irrigation did not seem to affect DO concentrations in the groundwater.

Groundwater pH

Groundwater pH in shallow wells was slightly acid throughout the study area (Table 3-16). Although statistical analysis showed that there were significant variations in pH across the field and over time, the total change was less than 1 pH unit. The pH tended to decrease towards the riparian zone, with lowest pH being observed in the shallow wells of riparian zone 2 (Table 3-16). The pH values in the shallow wells of riparian zone 2 did not show any seasonal variations.

Groundwater pH in the deep wells showed a similar trend as for the shallow wells (Table 3-17). More spatial and

Table 3-15. Change of dissolved oxygen (DO) concentrations in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Apr.	Jun.	Jul.	Sept.	Dec.
	mg L^{-1}				
upgrad	1.0aA‡	1.3cA	1.3bA	1.0aA	0.9aA
irrigated	0.9aAB	0.9bcAB	0.7aA	1.1aB	1.1aB
downgrad	0.6aA	0.6abA	0.6aA	1.3aB	0.9aAB
edge	0.9aBC	0.3aA	0.6aA	1.0aBC	1.2aC
riparian1	0.8aAB	0.5abA	0.5aA	1.1aB	1.2aB
riparian2	1.2aB	0.8abcAB	0.5aA	0.7aAB	1.1aB

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

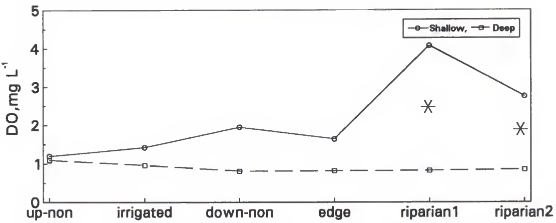

Fig. 3-12. Averaged groundwater dissolved oxygen (DO) concentrations in shallow and deep wells along the hydraulic gradient. The asterisks designate significant differences between the two depths.

Table 3-16. Change of pH values in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Dec.
	-------------------mg L^{-1}	--------------------		
upgrad	6.39bcAB‡	6.53cdB	6.27cdA	6.23cA
irrigated	6.54cdB	6.61dB	6.31dA	6.26cA
downgrad	6.62dC	6.41cB	6.00bcA	6.10cbA
edge	6.48bcdC	6.37bcBC	6.11bAB	6.20cB
riparian1	6.35bC	6.19bBC	6.07bAB	5.93bA
riparian2	5.80aA	5.81aA	5.73aA	5.66aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-17. Change of pH values in deep wells (180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Feb.	Mar.	Apr.	Jun.	Jul.	Sept.	Dec.
	----------	----------	----------	mg L^{-1}	----------	----------	----------
upgrad	6.32bcB‡	6.47bB	6.23bB	6.38bB	6.85cC	5.96cbA	6.27cB
irrigated	6.45cB	6.56bBC	6.15bA	6.15aA	6.61bC	6.06cA	6.20bcA
downgrad	6.47cC	6.44bC	6.11bB	6.01aB	6.54abC	5.76abA	6.10bcB
edge	6.49cCD	6.43bBC	6.05bA	6.00aA	6.70bcD	6.03cA	6.21bcAB
riparian1	6.20bBC	6.37bC	6.01bB	6.04aB	6.62bcD	5.60aA	6.01bB
riparian2	5.80aB	5.77aAB	5.71aAB	6.10aC	6.40aD	5.54aA	5.61aAB

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

temporal variations were observed in deep wells due to the larger number of measurements; however, the overall change in pH values was still small. Decreased pH values were found in deep wells of riparian zone 2 on most of the sampling dates. There were no significant differences between pH values of the shallow and deep wells throughout the entire study area (Fig. 3-13).

Groundwater temperature

Groundwater temperature varied only slightly along the hydraulic gradient for both the shallow and deep wells, even though the statistical analysis showed significant differences across both space and time (Tables 3-18, 3-19). The temperature increased after April, reached its highest point in September, and then decreased in December 1994. The range over which the groundwater temperature fluctuated between April and December 1994 was only 4 °C, however, even though the ambient air temperature evidenced much greater change during that period.

There was a significant decrease in water temperature for the deep wells in April; however, the change was only 1.2 °C (Table 3-20). The water temperatures were not different for the two depths in December.

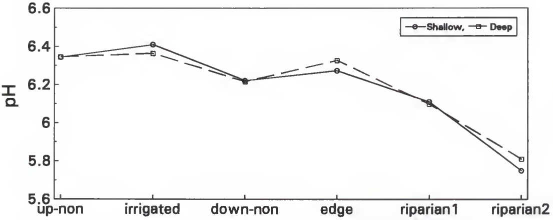

Fig. 3-13. Averaged groundwater pH values in shallow and deep wells along the hydraulic gradient. There was no significant difference between the two depths.

Table 3-18. Change of temperature in shallow wells (120 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Apr.	Dec.
	-------------mg L^{-1}-------------	
upgrad	23.0abB‡	20.5aA
irrigated	22.3abB	20.4aA
downgrad	23.4bB	20.8aA
edge	23.0abB	20.8aA
riparian1	21.9aB	21.0aB
riparian2	22.1abB	20.2aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-19. Change of temperature in deep wells a(180 cm) along the hydraulic gradient on different sampling dates (n=3).

Zone†	Apr.	Jun.	Jul.	Sept.	Dec.
	-----------------------------	-----------------------------	mg L^{-1} ------------------	-----------------------------	-----------------------------
upgrad	22.1cB‡	23.0bC	25.2cD	25.7bD	20.7abA
irrigated	21.4bB	22.2aC	24.9cD	25.8bE	20.4aA
downgrad	22.2cB	23.6bC	25.0cD	26.5cE	20.7abA
edge	22.0bcB	23.5bC	24.8bcD	25.8bcE	20.6abA
riparian1	20.3aB	22.1aC	24.1abD	25.3bE	21.3bA
riparian2	20.6aA	21.9aB	23.4aC	24.2aD	20.0aA

† Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

‡ Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and upper-case letters are for comparisons among dates within a given zone.

Table 3-20. Change of groundwater temperature with depth in April and December (n=3)†.

Depth†	April	December
	----------mg L^{-1}----------	
Shallow	22.6b‡	20.6a
Deep	21.4a	20.6a

† Numbers are averaged temperatures over all zones on each sampling date.

‡ Numbers followed by the same letters in the same column are not significantly different at the 0.05 level.

Soil Chemistry

Nitrate-N

The irrigated soils showed higher NO_3^--N concentrations in the top 20 cm of the soil profile on all sampling dates than did the other zones (Table 3-21). The downgradient surface soil (0-20 cm) also had higher NO_3^--N concentrations on most sampling dates. The surface soils in the riparian zone evidenced relatively low NO_3^--N concentrations.

Surface soils in the irrigated and downgradient zones evidenced higher NO_3^--N concentrations during summer time than during winter time (Table 3-21). The upgradient and riparian surface soils showed little temporal variation in NO_3^--N concentration, though high NO_3^--N concentrations were found in the field edge surface soil in September and then declined over time, reaching their lowest level in December. Temporal variations in NO_3^--N concentrations were less obvious for the subsurface soils.

Nitrate-N concentrations generally decreased with depth in the soil profiles of all zones (Table 3-22). However, NO_3^--N concentrations in the irrigated zone were generally higher than concentrations in the other zones at the lower depths in the soil profile.

Ammonium-N

Soil NH_4^+-N concentrations were generally low

Table 3-21. Nitrate-N concentrations in soil profiles along the hydraulic gradient (n=3).

Zone‡	June	July	September	December
	---------------- mg N kg^{-1}	-------------------		
--------------------------	Depth, 0-20 cm	-------------------		
upgrad	2.13aA†	7.42abB	5.32aAB	2.17aAB
irrigated	9.46bAB	10.08aA	12.48bA	6.04bB
downgrad	10.26bA	9.93aA	13.19bA	2.20aB
edge	3.68aAC	6.24bcA	10.35bB	1.70aC
riparian	1.32aA	3.08cA	4.02aA	1.99aA
--------------------------	Depth, 20-40 cm	-------------------		
upgrad	0.70aA	1.43aA	1.81aA	0.56aA
irrigated	2.96abAC	8.08bB	6.45bAB	1.01aC
downgrad	4.97bB	2.76aAB	3.88abAB	0.26aA
edge	2.76abAB	3.01aAB	5.48abA	0.42aB
riparian	0.23aA	1.22aA	1.09aA	0.23aA
--------------------------	Depth, 40-60 cm	-------------------		
upgrad	0.12aA	0.60aA	0.56aA	0.20aA
irrigated	0.68abA	5.50bB	1.85aA	1.75aA
downgrad	2.60bA	1.54aA	0.77aA	0.84aA
edge	1.71abA	1.92aA	1.02aA	0.46aA
riparian	0.11aA	0.64aA	0.54aA	0.15aA
--------------------------	Depth, 60-80 cm	-------------------		
upgrad	0.08aA	0.50aA	0.47aA	0.16aA
irrigated	0.42abA	2.34aAB	3.02bB	1.30abB
downgrad	2.27bA	0.76aA	0.60aA	2.59bA
edge	0.68abAB	0.85aAB	2.43abA	0.36aB
riparian	0.10aA	0.52aA	0.46aA	0.14aA
--------------------------	Depth, 80-100 cm	-------------------		
upgrad	0.06aA	0.51aA	0.51aA	0.13aA
irrigated	0.18aA	3.01bB	3.92bB	1.01aA
downgrad	2.01bAB	0.72aA	0.61aA	2.40bB
edge	0.48abA	0.62aA	1.61aA	0.40aa
riparian	0.12aA	0.51aA	0.48aA	0.15aA
--------------------------	Depth, 100-120 cm	-------------------		
upgrad	0.08aA	0.52aA	0.57aA	0.12aA
irrigated	0.08aA	2.79aA	8.49bB	2.57aA
downgrad	2.09aA	0.86aA	0.87aA	1.36aA
edge	0.62aA	0.52aA	0.85aA	0.48aA
riparian	0.35aA	0.52aA	1.78aA	0.14aA

† Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones for a given date, and upper-case are for comparisons among dates within a given zone.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Table 3-22. Changes in NO_3^--N concentrations of the soil profiles along the hydraulic gradient (n=3)†.

Depth (cm)	upgrad§	irrigated	downgrad	edge	riparian
	mg kg^{-1} N				
0-20	4.26a‡	9.52a	8.90a	5.50a	2.61a
20-40	1.04b	4.62b	2.97b	2.92b	0.69b
40-60	0.37b	2.44cd	1.44c	1.28c	0.36b
60-80	0.30b	1.77d	1.56bc	1.08c	0.30b
80-100	0.30b	2.03bd	1.44c	0.78c	0.32b
100-120	0.32b	3.48bc	1.29c	0.62c	0.70b

† numbers are averaged concentrations over all dates.

‡ Numbers followed by the same letters in the same column are not significantly different at the 0.05 level.

§ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

(< 2 mg kg^{-1}) throughout the study area. There was no significant difference in NH_4^+-N concentrations for surface soil among the zones on most sampling dates (Table 3-23). Relative higher NH_4^+-N concentrations in the surface soils of the irrigated, downgradient and field edge zones were observed in September.

When averaged over time, the NH_4^+-N concentrations were highest in the surface 20 cm of soil and decreased significantly at the lower depths in the upgradient, irrigated, downgradient, and field edge zones (Table 3-24). However, similar concentrations of NH_4^+-N were found in the upper 60 cm of the soil profile in the riparian zone, though the concentrations were still low. Ammonium-N concentrations were not significantly different among the zones below 80 cm in the soil profile.

Dissolved organic carbon (DOC)

The irrigated surface soils had higher DOC concentrations than did the soils in the other zones in September and December (Table 3-25). However, DOC concentrations in irrigated surface soils were not significantly different from those in other zones in June and July. Surface soils in the riparian zone also showed high DOC concentrations in July and September, but not in June and December. The higher DOC concentrations in the irrigated soil were also observed at all depths and for most

Table 3-23. Ammonium-N concentrations in soil profiles along the hydraulic gradient (n=3).

Zone‡	June	July	September	December
	mg N kg^{-1}			
Depth, 0-20 cm				
upgrad	1.05aAC†	0.45aB	0.80aAB	1.34aC
irrigated	0.91aA	0.57aA	1.58bB	1.03abA
downgrad	0.79abA	0.19aB	1.74bC	0.42bcAB
edge	0.33bcA	0.30aA	0.87aB	0.21cA
riparian	0.25cA	0.30aA	0.52aA	0.54cA
Depth, 20-40 cm				
upgrad	0.25aA	0.15aA	0.16aA	0.26aA
irrigated	0.16aA	0.12aA	0.58aA	0.44aA
downgrad	0.16aA	0.10aA	0.36aA	0.12aA
edge	0.12aA	0.11aA	0.21aA	0.11aA
riparian	0.12aA	0.88bB	0.14aA	0.48aAB
Depth, 40-60 cm				
upgrad	0.12aA	0.07aA	0.08aA	0.10abA
irrigated	0.07aA	0.10aAB	0.29aAB	0.40aB
downgrad	0.10aA	0.07aA	0.11aA	0.08bA
edge	0.06aA	0.05aA	0.08aA	0.10abA
riparian	0.13aA	0.56bB	0.07aA	0.20aba
Depth, 60-80 cm				
upgrad	0.07aA	0.05aA	0.08abA	0.08aA
irrigated	0.06aA	0.07abA	0.13aA	0.32bB
downgrad	0.07aA	0.05abA	0.05abA	0.08aA
edge	0.05aA	0.05aA	0.04bA	0.08aA
riparian	0.08aAB	0.14bA	0.05abB	0.16aA
Depth, 80-100 cm				
upgrad	0.05aA	0.04aA	0.06aA	0.09aA
irrigated	0.06aA	0.06aA	0.07aAB	0.13aB
downgrad	0.10aA	0.06aA	0.07aA	0.09aA
edge	0.05aA	0.04aA	0.04aA	0.09aA
riparian	0.08aA	0.05aA	0.04aa	0.15aB
Depth, 100-120 cm				
upgrad	0.08aA	0.04aA	0.05aA	0.06aA
irrigated	0.09aA	0.05aA	0.05aA	0.11aA
downgrad	0.08aA	0.05aA	0.04aA	0.07aA
edge	0.07aA	0.04aA	0.10aA	0.09aA
riparian	0.07aA	0.04aA	0.04aA	0.10aA

† Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones for a given date, and upper-case are for comparisons among dates within a given zone.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Table 3-24. Changes in NH_4^+-N concentrations of soil profiles along the hydraulic gradient (n=3)†.

Depth (cm)	upgrad§	irrigated	downgrad	edge	riparian
	---------------------mg kg^{-1} N---------------------				
0-20	0.92a‡	1.02a	0.78a	0.43a	0.40a
20-40	0.20b	0.33b	0.18b	0.14b	0.41a
40-60	0.09b	0.22bc	0.09b	0.07b	0.24ab
60-80	0.07b	0.14c	0.07b	0.05b	0.10bc
80-100	0.06b	0.08c	0.08b	0.06b	0.08bc
100-120	0.06b	0.07c	0.06b	0.08b	0.06c

† numbers are averaged concentrations over all dates.

‡ Numbers followed by the same letters in the same column are not significantly different at the 0.05 level.

§ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Table 3-25. Dissolved organic carbon concentrations in soil profiles along the hydraulic gradient (n=3).

Zone[‡]	June	July	September	December
	mg N kg^{-1}			
Depth, 0-20 cm				
upgrad	30.6aA[†]	39.9abA	40.4aA	27.8aA
irrigated	31.1aA	47.7acB	58.2bB	45.9bB
downgrad	23.8aAB	27.5bAB	37.8aA	19.3aB
edge	18.4aA	35.2abB	33.2aB	15.7aA
riparian	20.1aA	56.1cB	60.8bB	25.8aA
Depth, 20-40 cm				
upgrad	14.3aA	37.5aB	39.2aB	19.9aAB
irrigated	31.2aA	53.1aB	69.6bB	65.0bB
downgrad	18.1aA	34.2aAB	41.5aB	21.4aAB
edge	13.4aA	33.5aA	27.3aA	18.6aA
riparian	12.3aA	33.2aA	33.7aA	19.3aA
Depth, 40-60 cm				
upgrad	15.0abA	24.8aB	20.5aAB	17.8aAB
irrigated	26.6aA	51.5bBC	57.3bB	42.6bC
downgrad	18.7abA	26.5aA	20.1aA	19.1aA
edge	10.9bA	27.4aB	11.6aA	14.1aA
riparian	9.2bA	19.4aA	13.9aA	17.4aA
Depth, 60-80 cm				
upgrad	15.0aA	19.7abA	16.5aA	17.0aA
irrigated	16.3aA	41.0aAB	50.1bB	59.7bB
downgrad	16.5aA	24.2abA	14.2aA	12.3aA
edge	9.8aA	24.7abA	10.0aA	13.4aA
riparian	11.0aA	10.5bA	9.5aA	14.2aA
Depth, 80-100 cm				
upgrad	13.6aA	19.5aA	16.4abA	14.4aA
irrigated	10.7aA	24.8aB	22.3aAB	34.8bB
downgrad	14.3aA	19.7aA	11.2abA	12.3aA
edge	10.6aA	17.7aA	8.2bA	13.4aA
riparian	7.9aA	11.8aA	7.9bA	15.5aA
Depth, 100-120 cm				
upgrad	13.8aA	17.4aA	17.5aA	14.7aA
irrigated	13.2aA	17.9aA	16.6aA	29.9bB
downgrad	13.5aA	17.8aA	9.9aA	13.3aA
edge	11.9aA	16.5aA	9.5aA	12.3aA
riparian	10.5aA	11.2aA	9.0aA	12.4aA

[†] Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones for a given date, and upper-case are for comparisons among dates within a given zone.

[‡] Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

of the depths in December and September, respectively.

DOC concentrations decreased significantly with depth in the upper 60 cm of the soil profile, but showed little change below 60 cm in the upgradient, downgradient, field edge and riparian zones (Table 3-26). The DOC concentrations did not change in the top 40 cm of soil in the upgradient, downgradient and field edge zones. However, DOC concentrations decreased significantly for the second depth (20-40 cm) in riparian zone and increased significantly for the second depth in the irrigated soil. High DOC concentrations were found even at 80 cm in the irrigated soil profile.

Discussion

Groundwater Hydrology

The survey of soil surface elevation and groundwater table depth indicated that the study area consisted of two distinct subareas, both topographically and hydrogeologically. As a result, groundwater flow and subsequent nitrate transport in these two subareas were also different. The low groundwater flow rate in the flat "plateau" area of the field retarded nitrate movement in the groundwater, therefor enhancing the buildup of nitrate levels. The decrease of water head in the "slope" area, however, created a hydraulic gradient that served as a

Table 3-26. Changes in dissolved organic carbon concentrations of the soil profiles along the hydraulic gradient (n=3)†.

Depth (cm)	upgrad§	irrigated	downgrad	edge	riparian
			mg kg^{-1} N		
0-20	34.7a‡	45.7a	27.1a	25.6a	40.7a
20-40	27.7ab	54.7b	28.8a	23.2ab	24.6b
40-60	19.5bc	44.5a	21.1ab	16.0bc	15.0c
60-80	17.0c	41.8a	16.8b	14.5c	11.3c
80-100	16.0c	23.2c	14.3b	12.5c	10.8c
100-120	15.8c	19.4c	13.7b	12.5c	10.7c

† numbers are averaged concentrations over all dates.

‡ Numbers followed by the same letters in the same column are not significantly different at the 0.05 level.

§ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

driving force for groundwater flow from the field towards the downgradient riparian zone.

The development and movement of the NO_3^- contamination plume depend upon both the groundwater flow rate and its direction. The general decrease of groundwater table depth in a southeast-northwest direction resulted in a NO_3^- contamination plume which extended in a northwestern direction to the riparian zone at this study site.

Groundwater Quality

Nitrate-N

The elevated NO_3^--N levels in groundwater beneath the irrigated zone clearly indicated the impact of dairy effluent on water quality. Up to 61 mg L^- of NO_3^--N was found in this shallow groundwater, with averaged NO_3^--N concentrations in the irrigated groundwater on most sampling dates being greater than the EPA drinking water standard of 10 mg N L^{-1}. Elevated NO_3^--N concentrations in shallow groundwater have also been reported at other locations where dairy effluent (Andrews 1992) and other animal wastes such as poultry manure (Weil et al. 1990) have been applied. In a survey conducted over nine dairy farms in North Florida, Andrews (1992) found levels as high as 33 mg L^{-1} NO_3^--N in the shallow groundwater beneath effluent-irrigated croplands for a number of dairy farms.

Since the irrigated zone was not being supplied with any fertilizer N, the only source of N to the groundwater was from the effluent application. Once the effluent was applied to the soil surface, the NO_3 had to move through the soil column (vadose zone) before reaching the groundwater. The NO_3^--N concentration in the groundwater was the net result of the supply and consumption of NO_3^- in the soil profile.

Three criteria must be met in order for NO_3 to reach the groundwater (Johnson 1987): (1) NO_3^- must be present in excess of amounts required by plants and/or soil microorganisms; (2) the amount of rainfall or irrigation must exceed evapotranspiration in order to provide excess water for downward percolation; and (3) the soil must be sufficiently porous to allow water and NO_3^- to percolate. All three conditions were apparently met in this study. Continuous irrigation with N-rich effluent provided a sufficient amount of N at the soil surface. Accumulation of NO_3^--N in the groundwater coupled with excellent plant growth indicated that the amount of N supplied exceeded the amount needed by plants and soil microorganisms. The abundant rainfall in the area plus the effluent irrigation also provided sufficient water for downward leaching. The sandy soil above the groundwater table easily allowed the rapid percolation of water and NO_3^- to the groundwater.

In addition to irrigated-zone groundwater, high NO_3^--N concentrations were also found in the downgradient zone. This suggests an association of NO_3^--N and groundwater hydrology. The low hydraulic gradient within the "plateau" of the field resulted in apparent stagnation of groundwater beneath the field. However, the relatively steeper slope between the irrigated zone and the riparian zone created a higher hydraulic gradient and enhanced groundwater flow towards the riparian zone. The high NO_3^--N concentrations in the downgradient wells were apparently a result of "carry-over" of NO_3^--N from the upgradient irrigated-zone groundwater. The fact that NO_3^--N concentrations in the downgradient zone were not significantly different from those in the irrigated-zone groundwater suggested little change of environmental factors influencing the NO_3^--N concentrations in these two zones.

The significant decrease in NO_3^--N concentrations by the time the groundwater reached the edge of the field suggested that NO_3^- was removed from the groundwater between the downgradient zone and the edge of the field, even though this was only 60 meters in distance. The removal of NO_3^--N was evidenced by the decrease of NO_3:Cl ratios between these zones.

There are several mechanisms that could be responsible for NO_3^- removal in this groundwater system. Dilution could also reduce the NO_3^--N concentrations, by mixing the NO_3^--

contaminated groundwater with fresh water from other regions. Howard (1985) reported that dilution was the primary mechanism for lowering NO_3^--N concentrations in the Chalk aquifer of eastern-central England as NO_3^--contaminated groundwater was being discharged into the aquifer.

The chloride was considered as a conservative tracer in this study. The dilution factor based on the change of Cl^- concentrations between the downgradient zone and the edge of the field could only reduce the NO_3^--N concentrations by a factor of 1-1.5. The much lower NO_3^--N concentrations in the field-edge zone suggested that dilution was not playing a major role in reducing NO_3^--N in this groundwater. A possible explanation might be that the hydrogeological materials in this shallow aquifer was extremely homogeneous, so that the groundwater moved downgradient uniformly as a front allowing little mixing to occur.

Other possible mechanisms of NO_3^--N removal include microbial uptake, dissimilatory nitrate reduction to ammonium (DNRA) and biological denitrification. There is little information available on microbial population in the subsurface environment. It is generally agreed that microbial populations decrease significantly with soil depth (Yeomans et al. 1992). It can be reasonably assumed that microbial uptake cannot be a major NO_3^- removal mechanism in this particular case. The low NH_4^+-N concentrations in the groundwater (see following section) also suggest that DNRA was not a significant process in the NO_3^- removal. Exclusion of these processes implies that biological denitrification may be a significant mechanism responsible for NO_3^- removal from this shallow groundwater system (see later sections for further discussion).

The NO_3^--N concentrations in the irrigated-zone groundwater also showed seasonal variation. The NO_3^--N concentrations were highest in March and April of 1994 and generally decreased with time, reaching their lowest concentrations in December 1994. Kalita and Kanwar (1993) also reported a general decrease of NO_3^--N in groundwater with increased soil depths and over time during the growing season.

There was a close relationship between NO_3^--N concentrations in the groundwater of the irrigated zone and the amount of effluent applied through irrigation (Fig. 3-14). Extensive irrigation during the corn growing season (March to July) resulted in high NO_3^--N concentrations on the April and June sampling dates. Frequent irrigation in July and August produced another two NO_3^--N peaks on the August and September sampling dates. Due to the limited number of water sampling events, the immediate effect of irrigation on groundwater NO_3^--N levels could not be detected in January and October. However, the residual

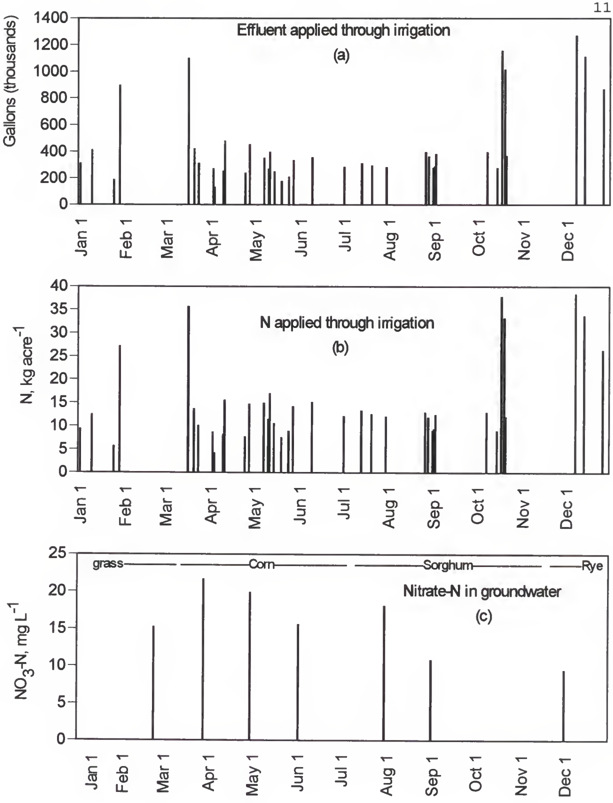

Fig. 3-14. The amounts of effluent (a) and N (b) applied through irrigation, and NO_3^--N levels (c) in the groundwater (deep) beneath the irrigated soil.

effect was still evident for the February and December samples.

Despite the occasionally higher NO_3^--N concentrations in irrigated shallow wells, there was no statistically significant difference between the shallow and deep wells. This suggests that NO_3^--N did not decrease significantly within the top 2-3 feet of groundwater. While many researchers have found a decrease in NO_3^--N concentrations with depth in the groundwater (Kalita and Kanwar 1993, Starr and Gillham 1993), the amount of NO_3^- attenuation below the water table in this study was not conclusive. This is likely due to the relatively small difference in depth between the shallow and deep wells in this case.

Ammonium-N

The major forms of N in the effluent used for irrigation were NH_4^+-N and organic N, with relatively little NO_3^--N (Table 3-27). Low NH_4^+-N concentrations in the groundwater indicated that NH_4^+ was either adsorbed on the soil particles or oxidized to NO_3^- during transport through the oxidized surface soil. Both adsorption and oxidation would prevent NH_4^+-N from leaching to the groundwater.

The significant increase of NH_4^+-N concentrations in the riparian zone could be an indication of DNRA. However, the process seemed to be limited since the total NH_4^+-N was still low. The limiting factor was probably the low supply

Table 3-27. Characteristics of effluent from the Dairy Research Unit.

Source	TKN	NO_3^--N	NH_4^+-N	DC	DIC	DOC	SRP	pH
	------------------	------------------	------------------	mg L^{-1}	------------------	------------------	------------------	
I	150	0.1	103	346	167	179	25	7.6
II	103	0.1	74	230	142	88	14	8.3

I = effluent from the primary lagoon, II = effluent from the retention pond.

of NO_3^--N. It has been hypothesized that competition between DNRA and denitrification processes is dependent on the ratio of carbon to electron (e^-) acceptors in the system studied (Tiedje et al. 1982). While low C:e^- ratios favor denitrification, DNRA would dominate in environments where the C:e^- ratio is high.

The difference in C and NO_3^--N concentrations between the irrigated groundwater and subsurface of the riparian zone suggests a change of the C:e^- ratios between these two environments. Although NO_3^--N concentrations decreased considerably from the irrigated zone to the riparian zone, the DOC concentrations were not significantly different in these two regions (Table 3-2, Fig.3-11). Therefore, the C:e^- ratios were greatly increased in the riparian groundwater, creating a favorable condition for DNRA. This hypothesis is supported by the work of King and Nedwell (1985) and Schipper (1991), who found that DNRA was favored when NO_3^--N concentrations were high in a salt marsh sediment and an organic riparian zone, respectively.

Dissolved organic carbon

Dissolved carbon data indicated that effluent irrigation significantly increased the DC and DOC concentrations in both shallow and deep wells of the irrigated zone. The last transect of shallow wells in the riparian zone also had high DC and DOC levels. However, the carbon sources in the two zones may have been different. High dissolved carbon concentrations in the effluent were apparently responsible for the elevated DC and DOC levels in the irrigated groundwater. Organic carbon in the riparian subsurface , on the other hand, may have been the result of long-term accumulation of organic matter in the forest soil. While the total contents were similar, the chemical compositions and bioavailability of the dissolved organic carbon in the two zones could be quite different due to differences in plant composition.

It appears that dissolved organic carbon in the effluent could percolate to the shallow groundwater, which fluctuated approximately between 50 and 140 cm below the irrigated soil surface. However, DC and DOC concentrations decreased significantly in the deep wells of the irrigated-zone and riparian groundwater. This decline of dissolved organic carbon below the groundwater table was also found by Starr and Gillham (1993) in a shallow water table aquifer in Canada. Starr and Gillham (1993) observed a sharp decrease in dissolved organic carbon (DOC) concentrations from about 10 to 1 mg L^{-} over a 1.25 to 2.5 m interval. They attributed the decline of DOC with depth to microbial utilization based on work at the same site by Trudell et al. (1986). The latter workers documented the presence of heterotrophic bacteria at the study site. It seemed that this hypothesis could also stand in this study. The long-term application of dairy effluent to the soil may have stimulated microbial activity in the subsurface environment.

Oxygen status

Dissolved oxygen (DO) concentration is a direct measurement of aerobic conditions in groundwater, and the level of DO has an immediate effect on the denitrification process in a groundwater system. Except for a few high DO readings, most DO concentrations in the shallow wells were less than 2 mg L^{-}. The DO concentrations in the deep wells were even lower, with most readings being less than 1.3 mg L^{-}. The lowest DO concentration found in the deep wells was below 0.4 mg L^{-}. Given that saturated DO contents at the temperatures found during this study range between 8.1 and 9.2 mg L^{-}, the groundwater seemed to be close to anaerobic if not totally devoid of oxygen. Although DO concentrations were only measured at two depths, the generally lower DO levels in the deep wells suggested a trend of decreasing DO with depth below the water table. This trend had been found in many other groundwater studies (Starr and Gillham 1993).

Irrigation with dairy effluent did not seem to affect DO concentrations in the groundwater, since the DO concentrations in the irrigated-zone groundwater were not significantly different from those in other zones.

Sources of DO in the groundwater include oxygen diffusion from the atmosphere via the overlying unsaturated soil, and infiltration of oxygenated water into the groundwater during either irrigation or rainfall events. Once the oxygen reaches into the groundwater, reduction of its DO is primarily through microbial respiration. This is often fueled by the existence of high DOC contents.

Oxygen is directly followed by NO_3^- in the microbialy mediated redox sequence. Theoretically, NO_3^- cannot be reduced if oxygen is present. However, the presence of anaerobic microsites in a soil environment could still allow denitrification to proceed under apparently oxidized conditions (Parkin 1987). The concurrent decrease of DO and NO_3^--N in groundwater has often been observed (Gillham 1991, Pedersen et al. 1991). Low NO_3^--N levels in a low DO environment clearly suggests the occurrence of denitrification. When examining an extensive DO-NO_3^- database, Gillham (1991) also found some high NO_3^--N concentrations in low DO groundwater samples. The high NO_3^--N and low DO levels in the irrigated groundwater of the present study provides another example of a low DO-high NO_3^--N situation. While Gillham (1991) did not provide any explanation, caution must be used when interpreting this type of observation.

Gillham and Cherry (1978) reported 2.0 mg L^- as being the upper DO limit for denitrification to proceed; however, this threshold value of DO may or may not be representative of other aquifer systems. Most of the DO concentrations in the groundwater of this study were below 2 mg L^{-} suggesting a conducive condition for denitrification. The presence of relatively high SO_4= concentrations in the irrigated groundwater also suggested that redox conditions was still high enough to prevent sulfate reduction.

Temperature and pH

The effluent used for irrigation tended to be alkaline, with pH values over 8 (Table 3-27). The pH levels in groundwater, however, were slightly acid beneath the irrigated area with average pH values around 6.4 in both the shallow and deep wells. Therefore, the alkalinity of the effluent was being neutralized in the soil column before reaching the groundwater. This was supported by the fact that lower pH was found in the soil profile of the study area (see soil chemistry section). Aerobic mineralization of organic carbon produces CO_2, which could also lower the pH by producing H_2CO_3 in both the soil profile and the groundwater.

Despite the spatial and seasonal variations, the pH in the groundwater beneath the cropped field was close to the optimum pH (6-8) for the denitrification process (Aulakh et al. 1992, Betlach and Tiedje 1981). Lower pH in the riparian-zone groundwater is generally believed to be due to the chemical composition of the plants in the forest, which produce acidic end products during microbial decomposition

(Davidson and Swank 1987).

Although reflecting the changes in ambient air temperature during the study period, water temperatures were constantly lower than corresponding air temperatures. The change of water temperature during the entire study was only 5 °C, suggesting that temperature should not have had a significant effect on the denitrification process.

Soil Chemistry

Nitrate and ammonium N

The effect of effluent irrigation on groundwater NO_3^--N was also reflected by the higher NO_3^--N contents in the soil profiles of the irrigated field (Tables 3-21, 3-22). It is hypothesized that NH_4^+-N in the effluent is being oxidized to NO_3^--N (nitrification) upon reaching the soil surface, with NO_3^- then percolating to the groundwater along with the downward movement of water. The higher NO_3^--N concentrations and relatively low level of NH_4^+-N (about 10 times lower than NO_3^--N) in the surface soil clearly support this hypothesis. Although the rate of nitrification is not known, the low level of NH_4^+-N on all sampling dates suggested a relatively rapid reaction rate.

Two zones of NO_3^--N accumulation were observed; one at the soil surface and the other in the groundwater. The oxidized condition and constant supply of NH_4^+-N during effluent irrigation seems to produce large quantities of

NO_3^--N at the soil surface. The negatively charged NO_3^- then leaches to the groundwater very easily. Once it reaches the groundwater, the NO_3^--N accumulates due to the low rate of removal.

Distribution of NH_4^+-N in the soil profile only showed one peak, at the soil surface. Furthermore, the highest NH_4^+-N concentration at the surface was 10 times lower than that of NO_3^--N despite the high NH_4^+-N content of the effluent. Adsorption of NH_4^+-N to negatively charged soil particles seemed to prevent NH_4^+-N from further downward movement.

Dissolved carbon

Unlike in the groundwater, where about half of the dissolved carbon was inorganic (Tables 3-10, 3-12), dissolved carbon in the soil solution tended to be dominated by organic forms. Therefore, the distribution of dissolved organic carbon followed closely the pattern of total dissolved carbon. Irrigation with the C-rich effluent resulted in higher DOC concentrations at almost every depth in the irrigated soil profile. While the 20-40 cm depth had the highest level of DOC, the 60-80 cm depth had almost the same concentrations of DOC as the top 20 cm depth. These high DOC concentrations in the lower soil profile apparently resulted from downward movement. The riparian soil also had high DOC concentrations; however, in this case high concentrations seemed to be limited to the top 40 cm of the soil profile.

Typical effluent and soil solution pH values were 8.3 and 6.0-6.3, respectively. Based on the chemical equilibria of a carbonate system (Snoeyink and Jenkins 1980), the inorganic carbon species should be dominated by HCO_3^- and H_2CO_3 within the pH range found in the present study. If that was the case, the negatively charged HCO_3^- should behave like NO_3^- and be subject to downward movement to the groundwater, resulting in higher DIC to DOC ratio in the groundwater than in the soil profile.

The DOC, however, behaved quite differently. Dissolved organic carbon can be retained by soil or aquifer materials through either physical adsorption (Jardine et al. 1989) or complex chemical reactions (Dunnivant et al. 1992). The high DOC concentrations in the subsurface (20-40 cm) soil suggested that the surface soil might have been saturated with DOC, allowing the excess DOC to move further down in the soil profile. A peak of DOC in the subsoil was also found by Zsolnay and Steindl (1991) at a typical agricultural soil. They observed that the water extractable organic carbon concentration from the 25-50 cm depth (roughly the B_t horizon) was about 9% greater than that for material from the A_p horizon, even though the total organic carbon concentration had declined by 57%. Dunnivant et al. (1992) concluded that the time required to saturate aquifer material with dissolved organic carbon was dependent on the influent DOC concentration with hydrophobic constituents of the DOC being preferentially adsorbed while hydrophilic components of DOC were rapidly transported through the aquifer.

Summary and Conclusions

This research investigated the effect of dairy effluent irrigation on both groundwater quality and soil chemistry in a poorly drained sandy soil at the Dairy Research Unit of the University of Florida, Gainesville, Florida. The study site included an irrigated cropped field and an adjacent forested riparian zone. Groundwater NO_3^- contamination, and its relation with the groundwater hydrology and to environmental conditions affecting NO_3^- removal (especially denitrification), in the soil profile were examined.

The dairy effluent was characterized by high NH_4^+-N and organic N levels along with alkaline pH, therefore acting as an N and C source to plants and microorganisms (including denitrifying bacteria) in both the soil and groundwater. Irrigation with such effluent on a highly permeable sandy soil site resulted in elevated NO_3^--N concentrations, to levels exceeding the EPA drinking water standard of 10 mg L^- in the groundwater directly beneath the irrigated soil. High NO_3^--N concentrations are also found in the downgradient groundwater, resulted from the downgradient flow of groundwater from the irrigated field towards the riparian zone. The NO_3^- disappeared between the downgradient zone and the edge of the field, limiting the NO_3^- contamination plume to a relatively small area. It is hypothesized that biological denitrification was mainly responsible for the NO_3^- removal in the groundwater, with dilution being less significant.

Irrigation with dairy effluent also increased total DOC in both the soil column and the groundwater. Deep penetration of the DOC could have fueled the denitrification process in deep portions of the soil profile as well as in the groundwater. The low DO concentration, near neutral pH and moderate temperature of the groundwater also suggested the conditions conducive for denitrification in this particular subsurface environment.

It was originally hypothesized that the riparian zone in this study site would be the critical zone for NO_3^- removal, based on the widely recognized functions of riparian systems. However, the fact that NO_3^- removal occurred primarily before the riparian zone was reached made the function of this particular riparian area in NO_3^- removal inconclusive.

CHAPTER 4

DENITRIFICATION IN DAIRY EFFLUENT-IMPACTED SANDY SOIL AND AN ASSOCIATED RIPARIAN ZONE

Introduction

Groundwater contamination with NO_3^- derived from agricultural practices has drawn considerable attention in recent years. The input of NO_3^--N into the groundwater not only threatens many important drinking water resources, but also cause eutrophication in surface water bodies such as lakes, streams and wetlands, if NO_3^- contaminated groundwater discharges to these surface water resources (Hallberg 1989, Heathwaite et al. 1993). Utilization of N-fertilizer and application of waste materials (including dairy manure and effluent) have been found largely responsible for NO_3^- contamination of groundwater in agricultural areas (Keeny 1989).

The number of dairy farms has been increasing greatly in North Florida over the last 20 years (Andrews 1992). These farmers have to deal with the large amount of manure and effluent produced in the operation. The preferred method of disposal of animal wastes is to recycle these materials as a replacement for fertilizer (Keeny 1989); therefore, applying dairy manure in the field and using the effluent for irrigation have become common practices on Florida dairy farms. However, extensive use of dairy effluent and manure on agricultural soils has raised concern about NO_3^- contamination of the shallow groundwater. An investigation by the US Geological Survey reported NO_3^--N concentrations exceeding the primary drinking-water standard of 10 mg N L^- in shallow groundwater wells beneath a variety of land uses (including effluent spray fields) for 13 dairy farms in North Florida (Andrews 1992, 1994). Isotope data has suggested that the NO_3^- in shallow groundwater wells originated either from animal wastes or a combination of synthetic fertilizer and animal wastes (Andrews 1994).

While identifying the pollution sources is important, the fate of NO_3^- derived from animal materials also needs to be studied in order to reduce potential contamination of the groundwater. Denitrification is one of the most important processes that can potentially remove NO_3^--N from the soil column and groundwater, therefore preventing inputs of NO_3^- to the groundwater.

Denitrification has been studied intensively with respect to N loss from agricultural soils, where this process is undesirable. In recent years, however, more research has been reported on denitrification as a favorable mechanism to remove NO_3^--N from NO_3^- contaminated environments. Denitrification in subsurface and riparian zone soils has been of particular interest during the last decade (Korom 1992, Lowrance 1992, Lowrance and Pionke 1989).

Although denitrification has been inferred in many instances by measurements of redox potential, DO concentration, NO_3^- disappearance, NO_3:Cl ratio (Gambrell et al. 1975, Jacobs and Gilliam, 1985, Simmons et al. 1992, Smith et al. 1976), few direct measurements of denitrification have been made on NO_3^- contaminated soil columns and shallow aquifers. The regulation of denitrification in subsurface environments is largely unknown.

A previous study at the Dairy Research Unit of the University of Florida found high NO_3^--N concentrations in the shallow groundwater beneath a cropped field irrigated with dairy lagoon effluent (see chapter 3). The NO_3^- disappearance in the downgradient non-irrigated zone, coupled with soil and water chemistry data, suggested favorable conditions for denitrification.

The objectives of this study were to (1) determine *in situ* denitrification rates in the different zones and different depths; (2) investigate the regulators of denitrification in the soil profile; and (3) evaluate the role of denitrification in removing NO_3^- from effluent-impacted soil. It is hypothesized that effluent irrigation should increase DOC concentrations in the soil column, therefore, enhancing denitrification in both surface and subsurface soils.

Materials and Methods

Study Site

This study was conducted at the Dairy Research Unit (DRU) of the University of Florida, about 19 km north of Gainesville, FL. The site consisted of a cropped field and adjacent riparian forest (Fig. 4-1). The crop field has been irrigated with dairy lagoon effluent using a center-pivot irrigation system for 5 years. Corn, sorghum and rye grass have been rotated in this field to provide either animal feed for the dairy farm or green cover. The riparian zone was located between the cropped field and a wetland in the downgradient forest. Native vegetation in the riparian forest included hardwood trees such as slash and longleaf pine and water and live oak. The understory consisted of a mixture of waxmyrtle, sumac, blackberry, gallberry, and scattered salpalmetto along with carpetgrass and other native weeds and grasses.

The soils in the cropped field were primarily Tavares Sand and Chipley Sand (thermic coated Aquic Quartzipsamments) and the soil in the riparian forest was Chipley Sand (thermic coated Aquic Quartzipsamments) (Thomas et al 1985). Although they were classified as ranging from moderately well drained (Tavares Sand) to somewhat poorly

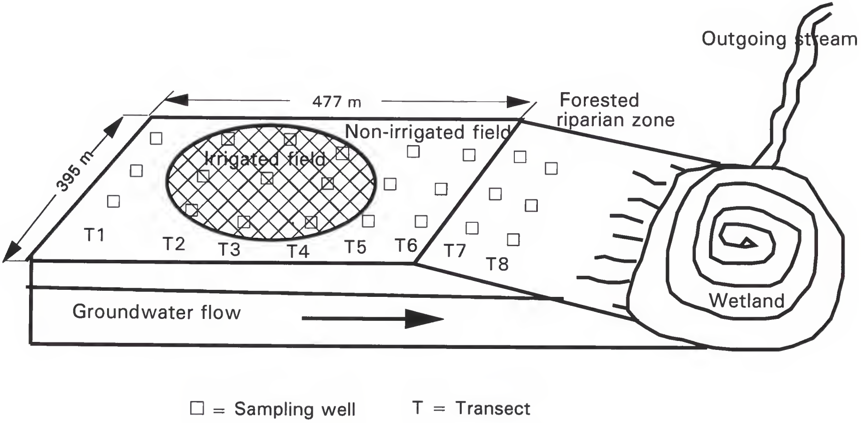

Fig. 4-1. Schematic diagram of the study site.

drained (Chipley Sand), the groundwater table was shallow,fluctuating between <20 and 200 cm in depth. Irrigation brought the water table even closer to soil surface during the wet season of each year. Due to the change of surface elevation from the cropped field to the wetland, the groundwater gradient was from the field, through the riparian zone, towards the wetland. Groundwater seepage reached the soil surface in the lower portion of the riparian zone.

Soil Sampling

In conjunction with a groundwater quality study (chapter 3), the study area was divided into five zones according to location along the hydraulic gradient and anticipated impact by NO_3^- in the groundwater. The five zones were identified as an up-gradient non-irrigated zone (UPGRAD), an irrigated zone (IRRI), a down-gradient non-irrigated zone (DOWNGRAD), the edge of the field (EDGE), and a riparian zone (RIPARIAN).

Intact soil cores were taken four times between June and December 1994, to a depth of 120 cm using an Environmentalists Subsoil Probe (Clements Assoc. Inc.). Four soil cores were taken in each zone. The intact soil cores were then transported back to the lab where they were sectioned at 20 cm intervals. Compaction was taken into consideration during the sectioning process. For each 20 cm interval, the depth between the top of the sampling probe and the soil surface both inside and outside the probe were measured, with the difference between these two depths reflecting the degree of compaction for that interval depth. For example, if the difference for the first 20 cm soil core was 2 cm, the compaction was 2 cm and only 18 cm of soil core was cut for the first 20 cm section. The soil cores then were covered with plastic caps at both ends and stored at 4°C prior to incubation. Soil moisture content was determined by drying approximately 20 grams of wet soil at 105 °C for 24 hours.

Denitrification Rates

Denitrification rates in the soil profile were determined using the acetylene inhibition technique (Tiedje et al. 1989) under both ambient and anaerobic conditions. After weighing, soil cores were transferred from the plastic liners into 5 cm i.d. plexiglass tubes equipped with butyryl rubber stoppers at both ends, for gas sampling. Transfer was done carefully so that the soil structure was kept intact. For ambient incubation, 10% of the headspace was replaced by acetylene, purified according to Hyman and Arp (1987) to remove contaminants. The headspace was mixed by pumping and releasing the syringe several times. The soil cores were then incubated at 25 °C for 6 hours, with gas samples being taken from the headspace 2 and 6 hours after the begining incubation. The headspace was also mixed well before each sample was taken.

Nitrous oxide (N_2O) in the gas samples was analyzed using a Shimadzu GC 14-A gas chromatograph equipped with a Ni^{63} electron capture detector set at 300 °C. The gas samples were separated on a 2 m column packed with Poropak Q. The oven temperature was set at 30 °C and the injector temperature set at 120 °C. The carrier gas was argon containing 5% methane with the flow rate being set at 30 ml min^{-1}. A molecular sieve was used to trap any contaminants from the carrier gas. Approximately 10 cm of the column at the injection end was packed with anhydrous $MgSO_4$ powder to trap moisture injected along with the samples (Schipper et al. 1993). The column was heated at 120 °C for 12 hours prior to use, to drive off any water vapor trapped in the $MgSO_4$. This resulted in good separation of N_2O from other components. The absolute amount of N_2O (mass) was used for all calculations, with 10% (v/v) N_2O gas (Scotty's Speciality) being used as a standard.

At the end of the incubation, the headspace volume was determined by measuring the pressure change before and after injecting 10 ml of air, using a pressure transducer. The headspace was then calculated using the Ideal Gas Law in the form of $P_1V_1 = P_2V_2$ (Parkin et al. 1984). Rates of denitrification were calculated based on the dry weight of soil used. Values were expressed as ng N_2O-N g^{-1} hr^{-1} unless otherwise stated.

Anaerobic denitrification rates were determined in the same way except that incubation tubes were flushed with pure N_2 gas for 5 min to create an anaerobic condition before acetylene was added.

Dissolved N_2O in the water was calculated based on the following equation by Tiedje (1982)):

$$M = C_g(V_g + V_1\alpha)$$

where M = the total amount of N_2O in the water plus gas phase,

C_g = concentration of N_2O in the gas phase,

V_g = volume of the gas phase,

V_1 = volume of the liquid phase,

α = Bunsen absorption coefficient, 0.544 for N_2O at 25 ^oC.

Denitrification Enzyme Activity (DEA)

Denitrification enzyme activity (DEA) is a measurement of the relative quantity of denitrifying enzyme present in a soil sample (Tiejde 1982), and should reflect the suitability of that soil for denitrification. Denitrification enzyme activity was determined using the acetylene inhibition method, coupled with the principle that rate is proportional to enzyme concentration when no other factors are limiting. Therefore, optimal conditions were used for incubation by providing NO_3^--N, organic carbon and an anaerobic environment.

Twenty (20) grams of field-moist soil were weighed into a 120 ml media bottle (Fisher Scientific) and treated with 20 ml of solution containing the following: 200 mg NO_3^--N L^{-1}, 2 g glucose-C L^{-1}, and 1 g chloramphenicol L^{-1}. Chloramphenicol was added to block the synthesis of any new denitrifying enzyme during the measurement period (Tiedje et al. 1989). After flushing with N_2 gas for 5 min, 10 percent of the headspace was replaced with purified acetylene and the mixture was incubated anaerobically for 3 hours, rotating at 100 rpm. Gas sampling and analysis then were done as for the denitrification study.

Data Analysis

Statistical analysis was done using SAS version 6.09 (SAS Institute Inc. 1992). Analysis of variance was carried out with the PROC GLM procedure based on a split-plot design. The Least Squares Means procedure was used to compare differences among the water quality and soil chemical parameters. All differences are reported as significant in a rejection region of $p \leq 0.05$.

Results

Denitrification under Ambient Conditions

Ambient denitrification rates were low in the surface 20 cm of soil, with a highest rate of only 2.75 ng N g^{-1} h^{-1} (Table 4-1). The surface soil in the irrigated zone had higher ambient denitrification rates than those from the edge and riparian zones in September; however, their rates were not statistically different from those in the upgradient and downgradient zones. Higher ambient denitrification rates in the irrigated surface soil were also observed in December, but they were only higher than those in the upgradient and down zones, not those in the edge and riparian zones. Ambient denitrification rates for the irrigated surface soils in June and July were similar to those found in the other zones. The surface soil in the downgradient zone showed significantly higher ambient denitrification rates than those of other zones in July; however, thess higher rates were not observed on any other sampling dates.

Ambient denitrification rates did not show any seasonal variations in the upgradient, irrigated, field-edge and riparian surface soils (Table 4-1). Surface soil in the downgradient zone had a peak rate in July, and lower rates on other sampling dates.

Table 4-1. Ambient denitrification rates in soil profiles along the hydraulic gradient (n=4).

Zone‡	June	July	September	December
	ng N g^{-1} h^{-1}			
Depth, 0-20 cm				
upgrad	0.05aA†	0.10aA	0.26abA	0.02aA
irrigated	0.94aA	0.57aA	1.47aA	1.40bA
downgrad	0.18aA	2.75bB	0.70abA	0.02aA
edge	0.06aA	0.06aA	0.06bA	0.09abA
riparian	0.01aA	0.24aA	0.11bA	0.08aba
Depth, 20-40 cm				
upgrad	0.01aA	0.04aA	0.10aA	0.01aA
irrigated	0.04aA	0.08aA	0.52abA	0.29aA
downgrad	0.04aA	0.37aAB	0.62bB	0.01aA
edge	0.01aA	0.03aA	0.05aA	0.04aA
riparian	0.00aA	0.18aA	0.08aA	0.01aA
Depth, 40-60 cm				
upgrad	0.02aA	0.03aA	0.10aA	0.01aA
irrigated	0.03aA	0.03aA	0.06aA	0.02aA
downgrad	0.03aA	0.17aA	0.62bB	0.00aA
edge	0.01aA	0.07aA	0.04aA	0.01aA
riparian	0.00aA	0.01aA	0.02aA	0.01aA
Depth, 60-80 cm				
upgrad	0.01aA	0.07aA	0.07aA	0.01aA
irrigated	0.02aA	0.08aA	0.06aA	0.03aA
downgrad	0.00aA	0.04aA	0.11aA	0.02aA
edge	0.00aA	0.08aB	0.04aAB	0.01aAB
riparian	0.07aA	0.04aA	0.07aA	0.03aA
Depth, 80-100 cm				
upgrad	0.01aA	0.04aA	0.17abA	0.01aA
irrigated	0.03aA	0.03aA	0.21aB	0.07aAB
downgrad	0.04aA	0.04aA	0.22aB	0.06aAB
edge	0.00aA	0.09aA	0.03bA	0.08aA
riparian	0.01aA	0.01aA	0.08abA	0.02aA
Depth, 100-120 cm				
upgrad	0.02aA	0.04aA	0.07aA	0.01aA
irrigated	0.07aA	0.07aA	0.64bB	0.25aAB
downgrad	0.13aA	0.81bB	0.14abA	0.08aA
edge	0.01aA	0.04aA	0.03aA	0.05aA
riparian	0.01aA	0.02aA	0.13abA	0.01aA

† Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and the upper-case letters are for comparisons among dates within a given zone.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Relatively higher ambient denitrification rates in irrigated and/or downgradient soils were only occasionally observed at lower soil depths in July and September (Table 4-1). The subsurface soils (below 20 cm) showed no differences in ambient denitrification rates among the various in June and December.

The mean ambient denitrification rate (over time) in the irrigated surface soil was significantly higher than at greater depths in the soil profile (Table 4-2). Although downgradient and irrigated zones had relatively higher ambient denitrification rates at a depth of 100-120 cm in July and September, respectively (Table 4-1), mean ambient rates at this depth in the downgradient and irrigated zones were not significantly different from those in the upper subsurface layers. Ambient denitrification rates in other zones showed no differences with depth.

Denitrification under Anaerobic Conditions

Denitrification rates measured under anaerobic conditions were much higher for the surface soils than the corresponding ambient rates (Table 4-3). The irrigated surface soil had higher anaerobic rates than for other zones in June, July and September. However, the surface soil denitrification rate in December was not significantly different from that of other zones. The anaerobic rates in surface soil of the downgradient zone were relatively high in summer time, and low in December. The riparian

Table 4-2. Changes in ambient denitrification rates of the soil profiles along the hydraulic gradient (n=4)†

	Depth, cm					
Zone‡	0-20	20-40	40-60	60-80	80-100	100-120
	ng N g^{-1} hr^{-1}					
upgrad	0.11A§	0.04A	0.04A	0.04A	0.06A	0.03A
irrigated	1.10A	0.23B	0.03B	0.05B	0.08B	0.26B
downgrad	0.91A	0.26A	0.20A	0.05A	0.09A	0.29A
edge	0.07A	0.03A	0.03A	0.03A	0.05A	0.03A
riparian	0.11A	0.06A	0.01A	0.03A	0.03A	0.04A

† Values are average concentrations over all dates.

§ Numbers followed by the same letters in a row are not significantly different the 0.05 level.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Table 4-3. Anaerobic denitrification rates in soil profiles along the hydraulic gradient (n=4).

Zone‡	June	July	September	December
	ng N g^{-1} h^{-1}			
Depth, 0-20 cm				
upgrad	5.23acA†	8.70acA	12.18acA	2.62aA
irrigated	20.63bAC	23.47bAB	32.63bB	10.96aC
downgrad	12.22abAB	12.99abcAB	18.17aA	3.40aB
edge	3.04acA	3.00aA	5.99cdA	2.91aA
riparian	1.26cA	14.29cB	0.63dA	1.89aA
Depth, 20-40 cm				
upgrad	0.03aA	0.46aA	0.18aA	0.08aA
irrigated	0.04aA	1.11aBC	2.05bB	0.71aAC
downgrad	1.66bA	2.31bA	1.84bA	0.09aB
edge	0.04aA	0.09aA	0.21aA	0.26aA
riparian	0.01aA	0.51aA	0.21aA	0.06aA
Depth, 40-60 cm				
upgrad	0.01aA	0.03aA	0.11aA	0.01aA
irrigated	0.01aA	0.13aA	0.30abA	0.10aA
downgrad	0.01aA	0.33aAB	0.48bB	0.01aA
edge	0.01aA	0.06aA	0.12abA	0.04aA
riparian	0.01aA	0.02aA	0.02aA	0.01aA
Depth, 60-80 cm				
upgrad	0.02aA	0.05aA	0.09aA	0.02aA
irrigated	0.01aA	0.10aA	0.10aA	0.03aA
downgrad	0.01aA	0.11aA	0.08aA	0.02aA
edge	0.01aA	0.07aA	0.07aA	0.02aA
riparian	0.01aA	0.06aA	0.64bB	0.02aA
Depth, 80-100 cm				
upgrad	0.02aA	0.01aA	0.16abA	0.02aA
irrigated	0.01aA	0.07aA	0.35aB	0.02aA
downgrad	0.01aA	0.12aA	0.17abA	0.03aA
edge	0.01aA	0.07aA	0.03bA	0.07aA
riparian	0.01aA	0.01aA	0.16abA	0.02aA
Depth, 100-120 cm				
upgrad	2.61aA	0.02aB	0.07aB	0.02aB
irrigated	0.13bA	0.06aA	1.05aA	0.04aA
downgrad	0.47bA	0.98aA	0.08aA	0.05aA
edge	0.02bA	0.05aA	0.03aA	0.85aA
riparian	0.01bA	0.02aA	0.47aA	0.01aA

† Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and the upper-case letters are for comparisons among dates within a given zone.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

surface soil had the same level of anaerobic denitrification rate as the downgradient zone in July. Upgradient, field-edge and riparian surface soils generally had low anaerobic denitrification rates on all sampling dates. Anaerobic denitrification rates for surface soils of the irrigated and downgradient zones were higher in the summer (June, July and September) than in the winter (December) (Table 4-3). The highest rates for the irrigated and downgradient zones appeared to be in September. Higher anaerobic denitrification rates in the irrigated and/or downgradient zones were also observed at 20-40 cm, with the peak rates at this depth occurring in July and September. However, this trend diminished at subsequently greater depths.

Unlike ambient denitrification rates, where only the surface soil in the irrigated zone showed higher rates than the associated subsurface soil, anaerobic denitrification rates in all zones were higher at the surface soil than at greater depths (Table 4-4).

Denitrification Enzyme Activity (DEA)

Denitrification enzyme activities for the surface 20 cm of soil showed no differences among the zones or dates except for September, when the irrigated surface soil had significantly higher DEA than the rest of the zones (Table 4-5). Higher DEA for the irrigated subsurface soil was

Table 4-4. Changes in anaerobic denitrification rates of the soil profiles along the hydraulic gradient.†

Zone‡	Depth, cm					
	0-20	20-40	40-60	60-80	80-100	100-120
	ng N g^{-1} hr^{-1}					
upgrad	7.18A§	0.19B	0.04B	0.04B	0.05B	0.68B
irrigated	21.92A	0.98B	0.14B	0.06B	0.11B	0.32B
downgrad	11.70A	1.48B	0.21B	0.06B	0.08B	0.40B
edge	3.74A	0.15B	0.06B	0.04B	0.05B	0.24B
riparian	4.52A	0.20B	0.02B	0.18B	0.05B	0.13B

† Values are average concentrations over all dates.

§ Numbers followed by the same letters in a row are not significantly different at the 0.05 level.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

Table 4-5. Denitrification enzyme activity concentrations in soil profiles along the hydraulic gradient (n=4).

Zone‡	June	July	September	December
	ng N g^{-1} h^{-1}			
Depth, 0-20 cm				
upgrad	26.5aA†	30.7aA	12.1aA	13.1aA
irrigated	33.2aA	41.2aA	132.1bB	35.1aA
downgrad	15.7aA	19.2aA	32.2aA	8.2aA
edge	11.5aA	11.2aA	27.1aA	7.7aA
riparian	17.1aA	19.8aA	29.7aA	12.7aA
Depth, 20-40 cm				
upgrad	0.5aA	0.3aA	3.3aA	0.1aA
irrigated	0.4aA	0.3aA	1.7aA	12.0bB
downgrad	0.2aA	0.2aA	1.7aA	0.2aA
edge	0.4aA	0.4aA	1.3aA	0.4aA
riparian	1.4aA	2.6aA	0.8aA	0.4aA
Depth, 40-60 cm				
upgrad	0.1aA	0.2aA	0.2aA	0.3aA
irrigated	0.2aA	0.1aA	0.4aA	0.1aA
downgrad	0.1aA	0.1aA	0.4aA	0.3aA
edge	0.4aA	0.4abA	0.4aA	0.2aA
riparian	0.2aA	0.8bA	0.4aA	0.4aA
Depth, 60-80 cm				
upgrad	0.4aA	0.4aA	0.3aA	0.2aA
irrigated	0.1aA	0.2aA	0.4aA	0.2aA
downgrad	0.2aA	0.1aA	0.4aA	1.8bB
edge	0.2aA	0.2aA	0.9aA	0.4aA
riparian	0.2aA	0.1aA	0.3aA	0.4aA
Depth, 80-100 cm				
upgrad	0.3aA	0.3abA	0.4aA	0.2aA
irrigated	0.1aA	0.1aA	0.3aA	0.2aA
downgrad	0.2aA	0.1abA	0.9bB	0.4aA
edge	0.1aA	0.1aA	0.5aA	0.4aA
riparian	0.5aA	0.5bA	0.6abA	0.5aA
Depth, 100-120 cm				
upgrad	0.4aA	0.5aA	0.2acA	0.2aA
irrigated	0.1aA	0.1abA	0.5abB	0.2aAB
downgrad	0.2aA	0.2abA	0.8bB	0.1aA
edge	0.0aA	0.0bA	0.5abB	0.2aAB
riparian	0.2aA	0.3abA	0.1cA	0.3aA

† Numbers followed by the same letters are not significantly different at the 0.05 level. The lower-case letters are for comparisons among zones on a given date, and the upper-case letters are for comparisons among dates within a given zone.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

observed only in December. Other depths generally showed no differences among zones.

Denitrification enzyme activities were higher in the surface soil (0-20 cm) than for the subsurface soils (below 20 cm) in all zones (Table 4-6).

Discussion

Denitrification Rates

Denitrification measured in this study exhibited a high degree of variability both in time and space. Coefficients of variation calculated across all zones, depth and sampling dates were 350, 306 and 332% for the ambient and anaerobic denitrification rates and DEA respectively. This high spatial and temporal variability for denitrification was also reflected in the skewed distributions of denitrification rates and DEA, with many low values and only a few high values (Fig. 4-2). Coefficients of variation for denitrification rates in agricultural soils have beenreported to range from 6% to 1800% in the literature (Aulakh et al. 1992).

The "hot spot" theory has been proposed to explain the high spatial variability of denitrification rates in soils (Parkin 1987). From a core segmentation experiment, Parkin (1987) found that 25-85% of the total denitrification activity of intact soil cores was associated with particulate organic material which represented only 0.08-

Table 4-6. Changes in denitrification enzyme activity of soil profiles along the hydraulic gradient (n=4).†

Zone‡	Depth, cm					
	0-20	20-40	40-60	60-80	80-100	100-120
	ng N g^{-1} hr^{-1}					
upgrad	20.62A§	1.05B	0.19B	0.33B	0.31B	0.32B
irrigated	60.43A	3.58B	0.21B	0.25B	0.18B	0.22B
downgrad	18.83A	0.59B	0.25B	0.61B	0.40B	0.30B
edge	14.39A	0.64B	0.35B	0.44B	0.27B	0.19B
riparian	19.86A	1.30B	0.49B	0.26B	0.52B	0.19B

† Values are average concentrations over all dates.

§ Numbers followed by the same letters in a row are not significantly different at the 0.05 level.

‡ Zone: upgrad = upgradient non-irrigated; irrigated = irrigated; downgrad = downgradient non-irrigated; edge = edge of the field; riparian = riparian zone.

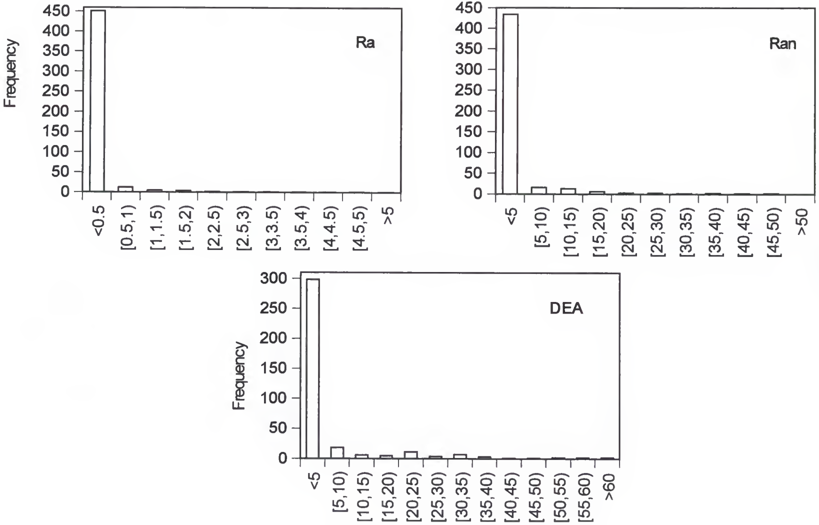

Fig. 4-2. Frequency distribution of ambient (Ra) and anaerobic (Ran) denitrification rates and denitrification enzyme activity (DEA) values measured during 4 sampling trips.

0.4% of the total mass of the soil cores. Only a few "hot spots" were found in four of 12 soil cores, suggesting that the patchy distribution of particulate organic material is a significant factor influencing the magnitude and variability of natural denitrification rates in soils. It is believed that the "hot spot" theory also applies to the present study. The presence of particulate organic materials in the soil not only provides necessary C source, but also enhances microbial respiration. This results in anaerobic microenvironments around the organic particles and allows denitrification to occur.

Whereas the patchy distribution of particulate organic materials can create "hot spots", the uneven distribution of dissolved organic carbon is also believed to be a factor influencing the variability of denitrification in soils. In the landscape of the present study, irrigation with dairy lagoon effluent in the cropped field apparently increased the heterogeneity of DOC distribution both across the landscape and with depth. This certainly could contribute to the high variability of denitrification in the present study.

Ambient denitrification rates were low in the surface soil (0-20 cm) throughout the entire study area. However, these rates were consistent with those reported in the literature from a variety of soil environments (Table 4-7). Although ambient denitrification rates for surface soil of

Table 4-7. Denitrification rates reported for different soil ecosystems.

Soil	Depth cm	Original rates reported	Adjusted rates† ng N g^{-1} h^{-1}	Reference
Agric. soil Canada	2.5-7.5	1.31 ng N g^{-1} h^{-1}	1.31	Bergstrom and Beaucham, 1993
Agric. soil Michigan	7.6	20-32 ng N g^{-1} d^{-1} prior to irrigation	0.8-1.3	Sexstone et al., 1985a
		209-383 ng N g^{-1} d^{-1} after irrigation	8.7-16	
Agric. soil Michigan	20	1.1 μg N kg^{-1} d^{-1} at 80% field capacity	0.04	Christensen et al., 1990
		32.5 μg N kg^{-1} d^{-1} at field capacity	1.4	
Riparian soil Georgia	10	1-2 μg N kg^{-1} d^{-1}	0.04-0.08	Ambus and Lowrance,1991
Temperate forest Michigan	15	164 g N ha^{-1} d^{-1} w.d. sand‡	3.0	Groffman and Tiedje, 1989
		409 g N ha^{-1} d^{-1} s.p.d. sand	7.8	
		187 N ha^{-1} d^{-1} p.d. sand	3.5	
		179 N ha^{-1} d^{-1} w.d. loam	3.3	
Agric. soil Florida	20	1.1 ng N g^{-1} d^{-1} effluent irrigated	1.1	This study
		0.07-0.09 ng N g^{-1} d^{-1} non-irrigated	0.07-0.91	
Riparian soil Florida	20	0.11 ng N g^{-1} d^{-1}	0.11	This study

† For comparison purpose, the reported denitrification rates were converted to ng N g^{-1} h^{-1} using an assumed 3000000 kg dry weight of soil for 20 cm-hectare.

‡ w.d. = well drained, s.p.d. = somewhat poorly drained, p.d. = poorly drained.

the irrigated zone were not significantly different from those in other zones, the trend is believed to be important. The constantly higher rates in the irrigated surface soil over all sampling dates suggested an effect of effluent irrigation on denitrification in the surface soil. The lack of statistical significance may be due to the high spatial variability of denitrification, which masked the effect of effluent irrigation on surface-soil denitrification. This was supported by the fact that there was a significant difference between ambient denitrification rates in the irrigated surface soil and those in non-irrigated soils, except for the downgradient zone, when using mean values over all sampling dates for analysis (Table 4-2).

The effect of effluent irrigation on denitrification in the surface soil was more clearly demonstrated by the anaerobic denitrification measurements. Anaerobic denitrification rates in the irrigated surface soils were often higher than those for upgradient, field-edge andriparian zones on most of the sampling dates (Table 4-3). The higher anaerobic denitrification rates in the downgradient surface soil cannot be explained using known environmental variables for the area.

Anaerobic denitrification rates were much higher their corresponding ambient rates, and there was less variability associated with them. Soil aeration appears to be extremely important in controlling denitrification under field

Although both ambient and anaerobic denitrification rates decreased dramatically with depth, measurable amounts of denitrification was still observed at lower depths in the soil profile (Tables 4-1, 4-3). The magnitude of denitrification found in the groundwater was similar to rates observed by Lowrance (1992) in a riparian subsoil of Georgia (approximately 0.3 ng N g^{-1} h^{-1}). Whereas low denitrification rates in the subsurface soils may not be significant compared to those of the corresponding surface soils, the long-term role of this denitrification activity with respect to NO_3^- removal from the subsurface soil, and especially from the groundwater, is not known.

Limiting Factors of Denitrification

The requirements for denitrification in the soil environment include the presence of denitrifying bacteria and NO_3^--N, the absence of O_2, an adequate supply of organic carbon, and suitable environmental conditions inclusing temperature and pH. Although these factors tend to be arranged in a hierarchical order with respect to their influence on denitrification (Davidson et al. 1990), it is important to realize that denitrification cannot occur if any of the conditions is not met. These factors are constantly changing with time and also vary from system to system. Denitrification in a particular soil environment can be totally inhibited by unfavorable conditions for any one conditions (Davidson and Swank 1986). In fact, many environmental variables, including water content, organic carbon, soil texture etc., exert an effect on denitrification through their controls on oxygen status of the soil. During anaerobic incubation the most important factor influencing denitrification, oxygen, is removed so that one of the key causes of variability is eliminated. This results in lower variability for the denitrification measurements.

Denitrification enzyme activity is a measure of the pre-existing denitrifying enzyme levels in a given soil. By providing the optimal conditions for denitrification, DEA values for different soils should only reflect the differences in functional denitrifying bacteria population. In theory, DEA measurements should have the smallest spatial variability; however, that was not true in the present study. The high spatial variability of DEA for the surface soils clearly suggested a highly variable distribution of denitrifying enzymes in the study area. The size of the bacterial population and the amount of functional denitrifying enzymes in the soil may be a result of long-term selection by environmental factors such as pH, nutrients, temperature and organic carbon (Schipper, 1991). Irrigation with C-and N-rich dairy lagoon effluent seems to provide or improve the conditions favorable to development of a denitrifying population in the surface soil.

factor and high denitrification rates can only be achieved when all factors are at optimal levels (Davidson and Swank, 1987).

The ambient denitrification rates in the surface soils were much lower than the anaerobic rates, clearly indicating the inhibitive effect of ambient conditions (O_2) near the soil surface (Fig. 4-3). Ambient denitrification rates in the irrigated and downgradient non-irrigated zones were only 5 and 8% of their corresponding anaerobic rates. The percentages for other zones were even lower, ranging from 1.5 to 2.4%.

Ambient conditions in the surface soils were evidenced by the high redox potentials in the upper portion of the soil profile (Table 4-8). Redox potentials of greater than 500 mV in both zones were well above the redox conditions required for biological denitrification in soils (generally considered to be less than 300 mV). However, the Eh is a measurement of mixed potential in a bulk soil, and anaerobic microsites in the soil may not be reflected in the soil Eh measurement. Measurable denitrification in the surface soils clearly suggested the existance of anaerobic microsites. We expected that effluent irrigation and rainfall events would limit oxygen supply in the surface soil shortly after water addition, thus promoting denitrification in the surface soil; however, the limited number of measurements of both redox potentials and denitrification rates may have

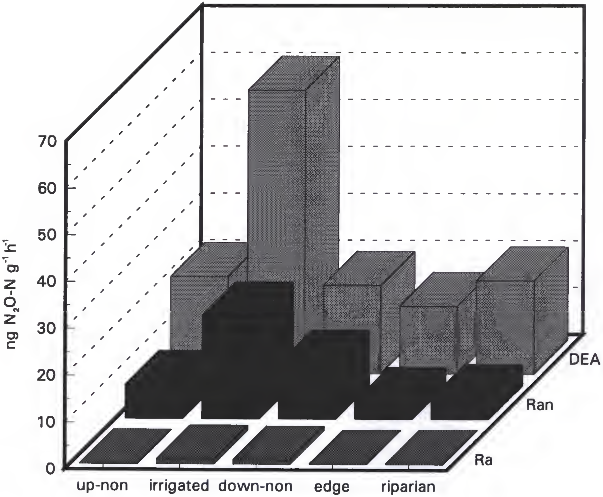

Fig. 4-3. Ambient (Ra) and anaerobic (Ran) denitrification rates and denitrification enzyme activity (DEA) values in the surface 20 cm of soil along the hydraulic gradient.

Table 4-8. Groundwater and soil characteristics in the study area†.

Depth cm	NO_3^--N irri‡	NO_3^--N non	DOC irri	DOC non	TC irri	TC non	DO irri	DO non	Eh irri	Eh non
Soil	mg kg^{-1}								mV	
0-20	9.5	8.9	45.7	27.1	15327	10310	-	-	537	575
20-40	4.6	3.0	54.7	28.8	9300	6270	-	-	581	600
40-60	2.4	1.4	44.5	21.1	5800	2257	-	-	586	603
60-80	1.8	1.6	41.8	16.8	4970	1460	-	-	563	557
80-100	2.0	1.4	23.1	14.3	2540	927	-	-	436	538
100-120	3.5	1.3	19.4	13.6	1803	743	-	-	283	384
Ground	mg L^{-1}						mg L^{-1}			
water	16.8	13.2	13.2	7.8	-	-	0.95	0.81	-	-

† Numbers for NO_3^--N, DOC and Eh are mean values over four sampling dates; TC concentrations are one time measurements; DO values in the groundwater are means over time for the deep wells.

‡ irri = irrigated zone; non = non-irrigated zone downgradient from the irrigated zone; except for Eh, where non = area upgradient from the irrigated zone.

prevented any decrease in Eh from being detected. Smith et al. (1976) measured the redox potentials in a cropped field and observed a sharp decrease in redox potentials at the 5 and 30 cm depths after the addition of potato processing waste water to the soil. Similarly Sexstone et al. (1985) found elevated denitrification rates in the surface 20 cm of a sandy soil immediately after irrigation, and observed the maximum rates within 3-5 hours. These results suggest that ambient conditions in the surface soil are a limiting factor for denitrification. Effluent addition to the surface soil could limit the supply of O_2, and therefore enhance denitrification. Based on these findings, we can assume that irrigation with dairy lagoon effluent could promote denitrification in the surface soil not only by providing a C source for denitrifiers but also by temporarily creating anaerobic micro-environments as well. This surge of denitrification in surface soils after irrigation may be important in removing NO_3^--N, even though the increase may be short lived (Smith et al. 1976).

High redox potentials persisted to a depth of 100 cm and dropped below 300 mV at depth of 120 cm in the irrigated soil profile (Table 4-8). However, redox potentials were still greater than 350 mv in the upgradient, non-irrigated zone. The decrease in redox potentials at greater depths in the irrigated soil suggested that the subsoil was more anaerobic and that the limiting effects of oxygen on denitrification would be less significant.

If Eh is not a definite indicator of soil aeration status, then the low DO concentrations in the groundwater should provide further supportive evidence for relatively anaerobic conditions at greater depths in the soil profile. The DO concentrations in the groundwater were less than 1 mg L^{-1} in both the irrigated and non-irrigated zones (Table 4-8). This dramatic decrease in DO concentrations (compared to the saturation point of 8-9 mg L^{-1} at the *in situ* temperatures) clearly suggested that the groundwater was anaerobic if not totally devoid of oxygen. However, the critical DO concentration at which denitrification occurs is not known. While Matthes (1982) reported that denitrification began at about 0.5 mg L^{-1} DO when OC was present in the groundwater, Gillham and Cherry (1978) reported that 2.0 mg L^{-1} was the upper DO limit for denitrification in a shallow aquifer of Canada. Apparently there is no single threshold value of DO for denitrification, and the more likely scenario would be that denitrification begins gradually within a moderate range of DO concentrations rather than at any single DO value.

In a survey of over 800 paired NO_3^- and DO values for shallow aquifers of Canada, Gillham (1991) reported that the data fell into 4 different categories which were closely related to the types of land use and texture of the soil.

Roughly 50% of data points fell within the low NO_3^--N (<1.5 mg L^{-1}) and low DO (<2 mg L^{-1}) category. Most of the these data were from cultivated and uncultivated fine-textured sites. Roughly 40% of the data were within the high DO and NO_3^--N category. This group of data were almost entirely from coarse-textured cultivated areas. Approximately 10% of the data were within the high DO and low NO_3^--N category. These samples were generally taken from sandy areas where there was little or no agricultural activity. Of particular interest, less than 1% of the data points fell within the category where high NO_3^--N was present in low DO groundwater. These samples were all taken from cultivated areas, with both fine and coarse-textured materials.

The coexistence of low DO and high NO_3^--N concentrations in shallow groundwater was also observed in the present study. The low DO concentrations suggested the consumption of DO in the shallow groundwater (<1.5 m) by microbial respiration. However there did not seem to be sufficient bioavailable DOC for the aerobes to deplete all DO in the groundwater. Therefore, denitrification may have been partially limited by residual DO in the groundwater.

Carbon limitations for denitrification have been reported in various types of soils were also observed in the present study. The DEA levels in the surface 20 cm of soils in all the zones were much higher than their corresponding anaerobic rates. This suggestes that denitrification in the surface soil may be limited by available organic carbon. Nitrate-N did not seem to be the limiting factor for denitrification in the surface soil since there was an ample supply of NO_3^--N (Table 4-8). The C limitation on denitrification in the surface soil seems to be less important than that by O_2, since the anaerobic rates were 22 to 62% of the DEA values, whereas the ambient rates were only about 0.5 to 5% of their corresponding anaerobic rates. Carbon limitation on denitrification in soils has been reported in many ecosystems including C-rich forest soils (Groffman et al. 1991) and riparian zones (Ambus and Lowrance, 1991). In fact, there are only a few cases reported where DEA was more or less fully expressed via high denitrification rates (Ambus and Lowrance 1991, Schipper et al. 1993).

The relative importance of O_2 and C on denitrification not only varies from system to system but also changes with soil depth. The O_2 limitation on denitrification in the surface soil was not observed in the subsurface soil, and especially in the groundwater (Fig. 4-4). Although anaerobic rates were higher than ambient rates in the subsurface soil, both were very low compared to those of surface soils. The low DEA in the groundwater suggested that denitrification was probably limited by the lack of viable denitrifying enzymes which could be the result of a lack of bioavailable organic carbon. Although total C contents in the soil

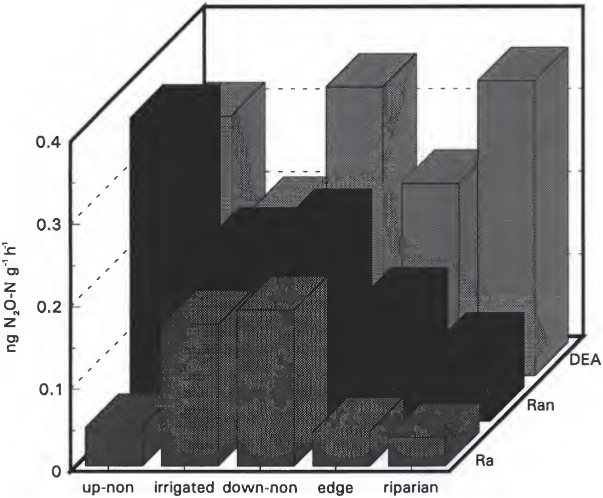

Fig. 4-4. Ambient (Ra), anaerobic (Ran) denitrification rates and denitrification enzyme activity (DEA) values at the 80-120 cm depth of soil profiles along the hydraulic gradient.

profiles were high, only a small portion was water soluble (DOC), and DOC concentrations decreased significantly in the groundwater (Table 4-8). Lack of a reliable method with which to measure the bioavailability of DOC to denitrifiers makes the values of DOC concentrations in the groundwater less meaningful. Apparently not all the DOC in the groundwater is available to denitrifiers, with low denitrification rates and DEA values for the groundwater supporting this hypothesis. In comparing denitrification rates for two riparian soils, Ambus and Lowrance (1991) observed higher denitrification potentials in the subsoil that had higher organic matter content. However, Groffman et al. (1991) observed strong stimulation of denitrification by glucose in forested soils with high organic matter contents, and little stimulation in grass plots which had lower organic matter contents. They concluded that the nature (quality) of the organic matter was more suitable for supporting microbial activity in the grass plots. Although effluent irrigation can increase DOC contents in the soil and groundwater, it is likely that the more available organic carbon was consumed by ambient respiration in the upper portion of the soil profile, with only the recalcitrant organic carbon being transported to greater depths and to the groundwater. Therefore, the ability of the DOC to support denitrifying activity was greatly reduced.

It is clear that irrigation with dairy lagoon effluent in this study supplied more NO_3^--N than the plants could assimilate, with the excess NO_3^--N being transported to the groundwater along with the percolation water. Nitrate-N accumulated in the groundwater beneath the irrigated soil due to the low denitrification activity of the groundwater. Groundwater monitoring has previously shown (Chapter 3) that flow of groundwater towards the riparian zone moved the NO_3^--N towards the non-irrigated zones (Table 4-8). However, the NO_3^- in the groundwater appears to have been depeleted before entering the riparian zone. Gilliam et al. (1974) reported a similar result for North Carolina coastal plain soils, i.e. lower concentrations of NO_3^--N in wells at the edges of the field than in the center of the field, with the groundwater flowing from the center to the edge. They suggested that this decrease was due to denitrification. Although low denitrification rates and DEA valueswere found in subsoils downgradient from the irrigated groundwater in the present study, these rates may still be significant because of the slow rate of groundwater movement. The groundwater flow rate was estimated to be only approximately 15 m yr^{-1} towards the riparian zone. Based on this rate, the time for the groundwater to travel from the edge of the irrigated area to the edge of the field could be as long as 4-5 years. It is likely that NO_3^--N in the groundwater would be gradually denitrified during this time period.

Denitrification rates and DEA in the riparian zone of the present study were low compared to those reported in the literature (Ambus and Lowrance, 1991, Schipper et al. 1993). Although the riparian soil had higher organic carbon content, the long term lack of NO_3^--N input to the system may have restricted the development of a denitrifying population in this soil.

Nitrogen Balance

Nitrogen balance is important for both efficient crop production and protection of the environment, especially in the sandy soils of Florida. Although calculation of the N balance in this study was complicated by a number of unknown factors, a simple balance sheet was developed for the irrigated zone (Table 4-9).

No fertilizer was applied to the irrigated field; therefore, the N input was totally from effluent irrigation. The amount of N applied through irrigation during 1994 was 657 kg ha^{-1}, which was the total amount of N input for the crop assuming negligible amount of N from rainfall and biological fixation. Approximately half of the N in the effluent from the retention pond was in NH_4^+-N form and half in organic-N form, with little NO_3^--N (Table 4-10). This N composition of the effluent is typical of that for many dairies in Florida (Gallaher et al. 1994).

Table 4-9. Nitrogen balance for the dairy effluent-irrigated crop field.

Budget	kg N ha^{-1} yr^{-1}
N input through irrigation†	657
N removal by crops‡	322
Residual soil NO_3-N	75
N removal by denitrification¶	
Ambient rate for 120 cm	41
Anaerobic rate for 120 cm	533
Ambient for top 80 cm	44
Ambient for top 100 cm	43

† Amount of effluent applied × TKN concentration

‡ Crop dry matter yield × N contents. Estimated from crop yields in a companion study (K. R. Woodard, personal communication).

¶ N loss through denitrification in a 120 cm soil column.

Table 4-10. Characteristics of effluent from the Dairy Research Unit.

Source	TKN	NO_3^--N	NH_4^+-N	TDC	TDIC	TDOC	SRP	pH
	mg L^{-1}							
I	150	0.1	103	346	167	179	25	7.6
II	103	0.08	74	230	142	88	14	8.3

I = Effluent from the primary lagoon, II = Effluent from the retention pond.

Not all the N in the effluent is available to plants when applied to the soil. Ammonium N is readily available for plant uptake, with this form of N accounting for as much as 50% of the N input through effluent irrigation (about 328 kg N ha^{-1} yr^{-1}). The rest of the N in the effluent was mainly in organic form which would only be available to plants after being mineralized to NH_4^+-N or NO_3^--N. Assuming that 25% of the organic N is available in the first year (Killorn 1993), the total amount of available N (NH_4^+-N + org-N) in the total N input (657 kg ha^{-1} yr^{-1}) would be 410 kg ha^{-1}.

The N removal processes in the effluent irrigation system include harvest as crops, denitrification, and ammonia volatilization. The amount of N removed by the corn-sorghum-rye multiple cropping system was 322 kg N ha^{-1} yr^{-1}, approximately 45% of the N applied through the effluent irrigation. Newton et al. (1995) also reported that over half of the N applied as liquid manure was removed by crops in the first year of application. This high recovery of N by crops clearly suggested the efficiency of recycling the nutrients in this type of effluent irrigation cropping system.

Whereas plant uptake can only proceed during the growing season, denitrification can occur all year long. Based on measured denitrification rates in a 120 cm soil profile, the estimated N loss through denitrification in the effluent irrigated field ranged between 41 kg N ha^{-1} yr^{-1} for totally ambient conditions to 533 kg N ha^{-1} yr^{-1} for totally anaerobic conditions (Table 4-9).However, totally ambient or anaerobic denitrification would not likely occur in the field. Assuming ambient rates for the top 80 or 100 cm of soil column and anaerobic rates below would not make much difference from the ambient rate for the whole soil column, since most of the denitrification activities were near the soil surface.

Irrigation has been shown to stimulate denitrification greatly in agricultural soils (Sexstone et al. 1985). Therefore, it is reasonable to expect that higher denitrification rates, close to anaerobic rates, could occasionally occur in response to the irrigation with C- and N-rich dairy lagoon effluent. If we assume that anaerobic rates occur for one out of seven days each week (the site averages one irrigation per week), N loss through denitrification could be as much as 118 kg N ha^{-1} yr^{-1}. This is consistent with the 133 kg N ha^{-1} yr^{-1} loss reported by Newton et al. (1995) in Georgia, where 348 kg of N was applied to a cropped field through dairy effluent irrigation using a center-pivot system. These findings clearly suggeste that denitrification is an important mechanism for N removal from effluent-irrigated cropping systems.

Nitrogen loss through NH_3 volatilization can also be a significant factor in the N balance. Hargrove (1988) reported that 20 to 90% of the N in soil could be lost through NH_3 volatilization. It was hypothesized that NH_3 volatilization would occur at a high rate during irrigation; however, the same concentrations of NH_4^+-N were found in the lagoon and the irrigation water collected during an irrigation, suggesting that NH_3 volatilization was not a significant loss during the irrigation process (Woodard, personal communication). Since NH_3 volatilization was not determined for the irrigated area, its contribution to N loss is not known for the present study. Nevertheless, significant amounts of N could be lost in this way.

Without considering NH_3 volatilization, the total input and output of N to the effluent-irrigated cropped field were 657 and 440 kg N ha^{-1} yr^{-1} respectively, resulting in a net gain of 217 kg N ha^{-1} yr^{-1}. It is important to realize that all not of all the N lost from the soil was necessarily from N applied in a given year, with some coming from N remaining from previous applications. As much as 67% of the N applied a given year could be removed, however, the net accumulation of N in the soil could serve as a N reserve for plant growth as well as a potential source of N contamination for groundwater.

Summary and Conclusions

Irrigation with dairy effluent is an economical means of wastewater disposal and nutrient management. Excess application of dairy effluent to sandy soils, however, has been shown to cause elevated NO_3^--N levels which often exceeding the drinking water standard in shallow groundwater. Denitrification was investigated at a poorly drained sandy soil irrigated with dairy lagoon effluent. Study objectives were to: (1) determine denitrification rates in the soil profile; (2) investigate the limiting factors for denitrification; and (3) evaluate the role of denitrification in protecting the shallow groundwater from NO_3^- contamination.

Ambient denitrification rates were low in the surface soil and decreased with depth. Anaerobic rates were much higher than corresponding ambient rates for the surface soils. The surface soils showed high denitrification potential (DEA), which also decreased dramatically with depth. The irrigated zone generally had higher denitrification rates and DEA compared to other zones, resulting from the long-term input of N and C through effluent irrigation.

The denitrification process was limited primarily by ambient conditions in the surface soils. Bioavailable C appeared to limit denitrification to a lesser extent. Denitrification in the lower depth of the soil profiles appeared to be limited by available C sources for denitrifiers. Although effluent irrigation increased DOC contents in the subsoils, ambient decomposition in the upper portion of the soil profile appeared to have consumed most of the readily available C allowing only recalcitrant C to leach to lower soil depths. Denitrification seemed to be responsible for N disappearance in the groundwater downgradient from the irrigated zone. Low inputs of NO_3^--N to the riparian soil may have inhibited the development of denitrifying populations, so the riparian zone in this study did not show the enhanced "N buffer" function that many riparian zone reportedly have.

Crop recovery was a major mechanism for N removal at this effluent-impacted site. About 50% of the N applied through effluent irrigation could be recovered the by crops. Denitrification was also important to N removal. Plant uptake and denitrification together could remove as much as 67% of the N applied through effluent irrigation in given year. This high recovery rate limited the amount of N leaching to the groundwater. However, the net gain of N in the soil suggests that excess N in the soil could serve as a potential source for groundwater contamination. Research on enhancing the denitrification process in the lower depths of the soil profile is needed.

CHAPTER 5
THE ROLE OF ORGANIC CARBON IN REGULATING DENITRIFICATION IN THE SOIL PROFILE

Introduction

Denitrification research remains a high priority, not only because of the need to reduce agricultural N losses, but also because of concern for NO_3^- contamination of groundwater. Nitrogen fertilizer and animal waste materials are the primary sources of NO_3^--N to the groundwater in agricultural areas (Keeny 1989). There are several factors that influence the resultant concentrations of NO_3^--N in groundwater: (1) the amount and source of N applied at the soil surface; (2) the amount of percolating water; (3) the permeability of the soil materials; and (4) the potential for denitrification (Hallberg 1989, Johnson 1987). Denitrification is the only process that can remove NO_3^--N from the soil or groundwater permanently; therefore, it has received considerable attention in the effort of reducing NO_3^- contamination of groundwater (Hiscock et al. 1991, Korom 1992).

Denitrification is a microbial process that requires anaerobic conditions, NO_3^- (an electron acceptor), and organic carbon (an electron donor) (Knowles 1982). These proximate regulators are in turn affected by many other physical, chemical and biological factors that are constantly changing both in time and space in the soil environment (Beauchamp et al. 1989, Tiedje 1988).

Organic carbon is one of the most important factors that affects denitrification in soils. However, the role of organic carbon in regulating denitrification is poorly understood due to the difficulty of measuring the availability of organic carbon and the complexity of the chemistry of organic carbon (Beauchamp et al. 1989).

There are many sources of organic carbon that can be used by denitrifying bacteria (Beauchamp et al. 1989). These carbon sources may exist in different forms such as plant residues, animal manure, etc. However, these substrates need to be water soluble before they are available to the bacterial population (McGill et al. 1986). Therefore, dissolved organic carbon (DOC) is considered as the most important factor influencing denitrification in the soil environment.

Plant residues and organic waste materials have been found to stimulate denitrification in the surface soil by providing both DOC and anaerobic conditions (Aulakh et al. 1984, deCatanzaro and Beauchamp 1985). However, there is little information available on the transport of DOC and its effect on denitrification in subsurface soil. In a recent study, McCarty and Bremner (1992, 1993) found that water extracts of surface soil promoted denitrification when added to subsoils, and that their ability to do so increased with increase in soil organic carbon content. They also reported that amendment of surface soils with corn and soybean residues increased the amount of organic C in aqueous extracts of these soils, and in the ability of these extracts to promote denitrification in subsoils.

In this study I investigated the effect of DOC in dairy lagoon effluent on denitrification for both surface and subsurface soils. It was hypothesized that the effluent can provide large quantities of DOC to promote denitrification in the surface soil when added through irrigation, and DOC in the surface soil can also increase denitrification in subsoil if transported to greater soil depths.

Materials and Methods

Soil Sampling

Soil samples were obtained from a cropped field at the Dairy Research Unit of the University of Florida. The center portion of the field has been irrigated with dairy lagoon effluent from the dairy farm for 5 years. The soil in the area was Tavares sand (thermic coated Aquic Quartzipsamments) (Thomas et al. 1985). Annual rotations of corn, sorghum and rye grass were used to provide either animal feed for the dairy farm or green cover. Six soil cores were taken from irrigated and non-irrigated areas to 120 cm, using an Environmentalists Subsoil Probe (Clements Assoc. Inc.). Six subsamples from each area, corresponding to the 0-20 and 80-120 cm depth intervals, were mixed and the bulk samples were passed through a 2 mm screen. They were stored at 4 °C. A total of 4 soil samples were used in the study: non-irrigated surface soil (NON 0-20), irrigated surface soil (IRRI 0-20), non-irrigated subsoil (NON 80-120) and irrigated subsoil (IRRI 80-120).

Soil Carbon Extraction

Water extracts of surface soils and effluent-treated surface soils were obtained for this study. Water soluble organic carbon from non-irrigated and irrigated surface soils was extracted from field-moist samples (100 g dry wt.) with sufficient water to bring the total volume of water to 100 ml. The soil-water suspensions were shaken for 1 hour and centrifuged at 9,268 x g. The supernatant liquids then were filtered through a 0.2 μm membrane filter to remove suspended soil particles and microorganisms (McCarty and Bremner 1993). The filtrate was analyzed for organic carbon using a high temperature Dohrmann DC-190 TOC Analyzer.

Extracts following effluent amendment were obtained in a similar manner. Effluent was obtained from the primary lagoons (EFFLUENT I) and the retention pond (EFFLUENT II) at the dairy farm. Effluent from both anaerobic lagoon and retention ponds have been used for irrigation in Florida (Gallaher et al. 1994). Field-moist soil (100 g dry wt.) from non-irrigated and irrigated surface soils were treated with 85 ml of the effluent (I and II), and water then added to bring the total amount of water to 100 ml. The effluent to soil ratio (1.2:1) used for the extraction was close to the effluent to soil ratio for each irrigation event, assuming 3 million kg of dry soil per hectare for a 20 cm depth. The mixture was shaken on a mechanical shaker for 1 hr and centrifuged at 9,268 x g. The supernatant was then filtered through a 0.2 μm membrane filter to remove soil particles and microorganisms.

Denitrification Capacity

Field moist soil samples (20 g dry wt) were weighed into 120 ml media bottles equipped with butyryl stoppers and stopcocks allowing gas sampling and flushing. To determine denitrification rates under non-limited NO_3^--N conditions, 2 mg of NO_3^--N was added to each bottle using a concentrated NO_3^--N solution. Water was then added to each bottle to bring the water content to a total of 10 ml. After flushing with purified N_2 gas for 15 minutes, 10% of the headspace was replaced by C_2H_2 purified according to Hyman and Arp (1987) to remove the contaminants present in the commercial C_2H_2 gas. Soil samples from irrigated and non-irrigated areas at both depths were used for this test. Three replicates were used for each soil sample. A treatment to which no NO_3^--N was added was also included for each soil, as a control. The bottles were incubated at 25 °C with periodical shaking, with Gas samples being taken at 1, 3, 7, 10 days and stored in Vacuutainers for subsequent analysis.

Denitrification Potential

Denitrification potential was determined under non-limited NO_3^--N and C conditions. The test was done in the same way as the denitrification capacity test, except that 20 mg of glucose C was added to each bottle along with 20 mg of NO_3^--N. A treatment with only addition of 20 mg of NO_3^--N was used as a control. This test was also conducted for soils from both the irrigated and non-irrigated areas, at both depths.

Effluent Effect on Surface Soil Denitrification

To evaluate the effectiveness of dairy waste effluent as a C source for denitrification in the surface soils, field moist soil samples (20 g dry wt.) from both irrigated and non-irrigated areas were weighed into 120 ml media bottles equipped with butyryl stoppers and stopcocks allowing gas sampling and flushing. Ten (10) milliliters of effluent I or effluent II were added to each bottle, along with 20 mg of glucose C in two ml of water. The soil suspensions were incubated for 10 days in the same way as for the denitrification potential study. Nitrous oxide production was analyzed after 1, 3, 7 and 10 day incubation periods. A control treatment with 10 ml of water and 20 mg of NO_3^--N was included for each soil, and three replicates were used for each treatment.

Carbon Source Effect on Subsoil Denitrification

The effect of water extracts of surface soils and effluent-treated surface soils on denitrification in subsurface soils (80-120 cm) was studied in the following way: Field moist soil samples (20 g dry wt.) were weighed into 120 ml media bottles. Ten ml of the water extracts of either surface soils or effluent-treated surface soils were added to each bottle, along with 20 mg of NO_3^--N in 2 ml of water. Water extracts of surface soils were only added to their corresponding subsoils, i.e. irrigated to irrigated and non-irrigated to non-irrigated. A control treatment was included for each soil, with only 10 ml of water and 2ml of NO_3^--N solution containing 20 mg of N. The treated soil suspensions were incubated as described previously.

Nitrous Oxide Analysis

Nitrous oxide (N_2O) in the gas samples was analyzed using a Shimadzu GC 14-A gas chromatograph equipped with a Ni^{63} electron capture detector set at 300 °C. The gas samples were separated on a 2 m column packed with Poropak Q packing material. Oven temperature was set at 30 °C and injector temperature was set at 120 °C. The carrier gas was argon with 5% methane, at a flow rate of 30 ml min^{-1}. A molecular sieve trap was used to remove any contaminants in the carrier gas. About 10 cm of the column at the injection end was packed with anhydrous $MgSO_4$ powder to trap any moisture injected along with the samples (Schipper et al. 1993). The column was heated at 120 °C for 12 hours prior to use, to drive off water vapor trapped in the $MgSO_4$ and thus produce a good separation of N_2O from other components. The absolute amount of N_2O (mass) was used for calculation with 10% (v/v) N_2O gas (Scotty's Speciality) being used as an standard.

Dissolved N_2O in the water was calculated based on the following equation, by Tiedje (1982):

$$M = C_g(V_g + V_l\alpha)$$

where M = total amount of N_2O in the water plus gas phase,

C_g = concentration of N_2O in the gas phase,

V_g = volume of the gas phase,

V_l = volume of the liquid phase,

α = Bunsen absorption coefficient, 0.544 for N_2O at 25 °C.

At the end of the incubation the headspace volume was determined by measuring the pressure changes before and after injecting 10 ml of air, using a pressure transducer. The headspace was then calculated using the Ideal Gas Law in the form of $P_1V_1 = P_2V_2$ (Parkin et al. 1984). The N_2O

production was calculated based on the dry weight of soil used and expressed in μg N_2O-N g^{-1} for the time specified.

Results

Denitrification Capacity

The amount of N_2O-N produced in the non-irrigated surface soil with NO_3^- added showed a linear increase over a 10-day period (Fig. 5-1). The control treatment (no NO_3^- addition) showed little change over the amount of N_2O-N produced in the same period.

A linear increase in N_2O production was also observed for the irrigated surface soil (Fig. 5-1). However, the total amount of N_2O produced during the 10 day incubation period was approximately twice that for non-irrigated soil. In contrast to the non-irrigated soil, the control treatment for the irrigated soil produced N_2O during the 10 day incubation period, with higher N_2O production in the NO_3^--N treated soil being observed only after 3 days incubation. At the end of the incubation, the amount of N_2O produced in the NO_3^--N treated irrigated surface soil was more than twice that for the control.

Although the subsoils from both irrigated and non-irrigated areas also showed increased N_2O production upon addition of NO_3^-, the amount of N_2O-N produced in a 10-day period was much smaller than for the corresponding

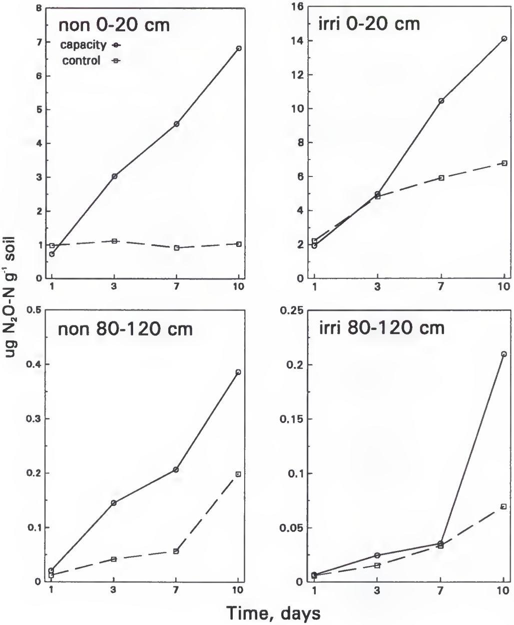

Fig. 5-1. N_2O production over time for surface and subsoils treated with NO_3^--N (denitrification capacity) and water (control).

surface soils (Fig. 5-1). The total amounts of N_2O-N produced after the 10 day incubation of the non-irrigated and irrigated subsoils were only 6 and 1.5% those of their corresponding surface soils. The control treatment for the non-irrigated subsoil also showed an increase in N_2O production, producing about half the amount which the NO_3^- treatment produced by the end of the 10-day incubation. Similar to the surface soil, irrigated subsoil produced as much N_2O as the NO_3^--N treatment had during the early incubation period. However, the total amount of N_2O produced in the control after 10-day incubation was only one third that of the NO_3^--N treatment. The amount of N_2O-N produced in the non-irrigated subsoil was about twice that of irrigated subsoil.

Denitrification Potential

Denitrification potentials (following the addition of both NO_3^--N and C) for both irrigated and non-irrigated surface soils were much higher than their corresponding denitrification capacities (with only addition of NO_3^--N) (Fig. 5-2). The amount of N_2O-N produced by the end of the 10-day incubation was similar for irrigated and non-irrigated surface soils. However, potential N_2O production was 25 times higher than the denitrification capacity for the non-irrigated surface soil, and 13 times higher than for the irrigated surface soil. The rate of potential N_2O

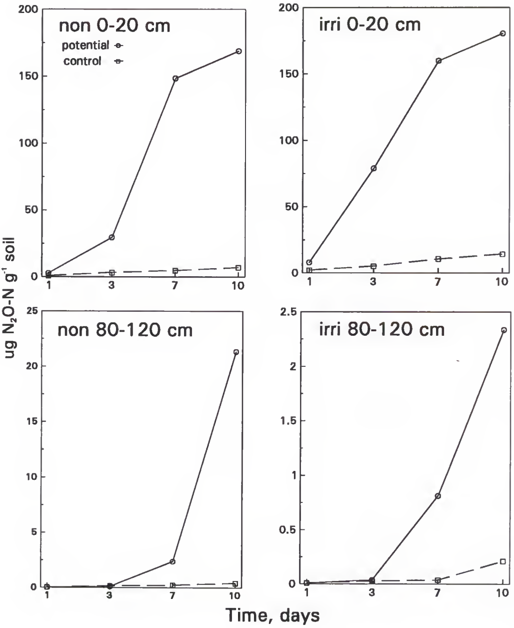

Fig. 5-2. N_2O production over time for surface and subsoils treated with NO_3^--N and C (denitrification potential) and NO_3^--N only (control).

production in both irrigated and non-irrigated surface soils decreased after 7 days of incubation.

The potential N_2O-N production in irrigated and non-irrigated subsoils was much lower than for the corresponding surface soils (Fig. 5-2). However, potential N_2O-N production was significantly higher than that in capacity treatments for both subsoils. Potential N_2O-N production in the subsoils did not level off during the last three days of incubation as it did for the surface soils. The subsoils, on the other hand, did not show any response to the addition of NO_3^--N until after three days of incubation (Fig. 5-2). The total amount of potential N_2O-N produced for the non-irrigated subsoils was about 10 times that for the irrigated subsoil, even though the denitrification capacities were similar for the two subsoils.

Effluent Effect on Denitrification in Surface Soils

Irrigated and non-irrigated surface soils treated with effluent I produced more N_2O-N than did the control soils after 1 day of incubation (Fig. 5-3). The N_2O production for these effluent-treated surface soils was even higher than the "potential" N_2O production (Fig. 5-2) after 1 day of incubation. The production of N_2O for the first 7 days was linear and then leveled off, similar to the potential N_2O production for the surface soils (Fig. 5-2). The total amount of N_2O produced by the surface soils treated with

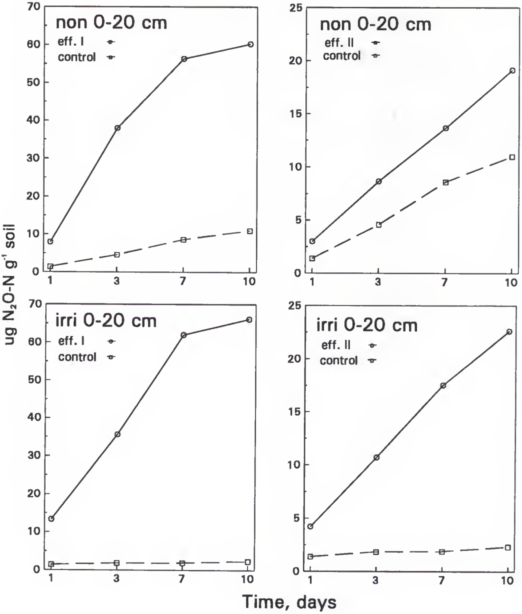

Fig. 5-3. N_2O production over time for surface soils treated with NO_3^--N and effluent (eff I and eff II) and NO_3^--N only (control).

effluent I after a 10-day incubation was 5 and 14 times higher than that of the control for non-irrigated and irrigated soils, respectively. The control treatments had higher N_2O production for the non-irrigated soil than for the irrigated soil.

Surface soils treated with effluent II showed a linear increase in N_2O-N production within the 10-day incubation period (Fig. 5-3). However, N_2O-N production was much lower than for soils treated with effluent I. The total amount of N_2O-N produced for non-irrigated and irrigated surface soils treated with effluent II was only 31 and 34% of that produced by corresponding soils treated with effluent I. Although N_2O production was similar for both irrigated and non-irrigated surface soils treated with effluent II, the non-irrigated soil showed greater response to effluent II treatment (compared to the control) than did the irrigated soil. Again, the leveling off of N_2O production by the end of the incubation (observed for effluent I treatments) was not noted for the effluent II treatments.

Carbon Source Effect on Subsoil Denitrification

Nitrous oxide production in non-irrigated subsoil treated with water extract from the surface soil did not show a significant difference from the control (Fig. 5-4). Water extracts from the irrigated surface soil, however, exhibited a stimulatory effect on denitrification for the

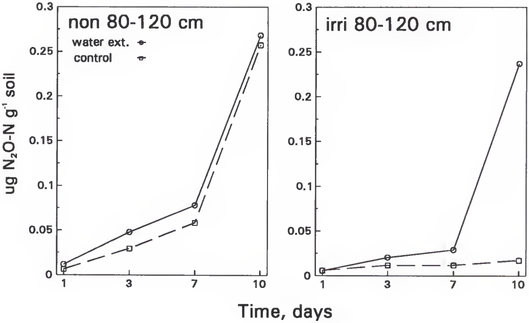

Fig. 5-4. N_2O production over time for subsoils treated with NO_3^--N and water extracts of corresponding surface soils (water ext.) and NO_3^--N only (control).

irrigated subsoil. The N_2O production for water-extract treated non-irrigated subsoils was similar to that for irrigated subsoils.

The extracts of effluent-treated surface soils showed a significant stimulatory effect on N_2O production in subsoils (Fig. 5-5). However, the effect was different for each soil and for each effluent. The greatest N_2O production was observed for non-irrigated subsoil treated with effluent I extract. The N_2O production was less in the irrigated subsoil, and non-irrigated and irrigated subsoils showed a leveling off of N_2O production (plateau) after 3 or 7 days incubation, respectively.

Although non-irrigated subsoil treated with effluent II extract produced more N_2O than did irrigated subsoil, total N_2O production for both soils treated with effluent II extracts was smaller than for their corresponding effluent I treatments (Fig. 5-5). Unlike the effluent I extract treatments, the effluent II treatments still showed a linear increase in N_2O production during the later portion of the incubation period. The control treatments for the irrigated subsoils showed little N_2O production during the 10-day incubation period. The control for non-irrigated subsoil produced more N_2O than did the irrigated soil, however, and the production was much lower than for the effluent treatments.

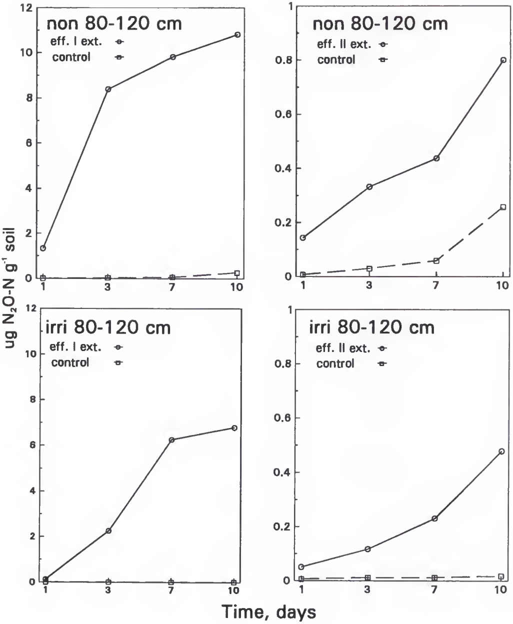

Fig. 5-5. N_2O production over time for subsoils treated with NO_3^--N and water extracts of effluent treated surface soils (eff I ext. and eff II ext.) and NO_3^--N only (control).

Discussion

Denitrification Capacity and Potential

The linear increase of N_2O-N production for the non-irrigated surface soil suggested that this soil has a significant denitrification capacity. The low N_2O production for the control suggested that denitrification in this soil might be limited by the supply of NO_3^-. The non-irrigated surface soil had a NO_3^--N concentration of only 1.3 mg kg^{-1}, corresponding to 26 μg NO_3^--N in the 20 g of soil used (Table 5-1). Approximately 77% (20 μg) of this NO_3^--N was denitrified in the first day of incubation. Insufficient supply of NO_3^--N apparently resulted in the low denitrification rates subsequently. When excess NO_3^--N was supplied, as in the denitrification capacity test, N_2O production was sustained at a rate of approximately 0.03 μg N_2O-N g^{-1} hr^{-1} for as long as 10 days. This prolonged denitrification rate clearly indicated that the C in the non-irrigated surface soil can support significant denitrification activity under anaerobic conditions.

The higher N_2O-N production for the irrigated surface soil suggested that more favorable conditions for denitrification then in the non-irrigated soil. Denitrification rates in the irrigated surface soil treated with NO_3^--N averaged approximately 0.06 μg N_2O-N g^{-1} hr^{-1} during the 10 day incubation period, twice that for the non-

Table 5-1. Characteristics of the lagoon effluent and water extracts of soils and effluent-treated surface soils used in this study.

Soil	Depth	Extract†	pH	NH_4^+-N	NO_3^--N	DC	DIC	DOC
Surface Soil				---------------mg L^{-1}---------------				
non	0-20	water ext.	5.7	0.5	0.6	15.6	1.0	14.6
non	80-120	water ext.	5.6	0.04	0.05	7.6	0.3	7.3
irri	0-20	water ext.	5.9	1.3	3.9	23.8	1.9	21.9
irri	80-120	water ext.	5.7	0.04	0.2	6.7	0.3	6.4
Effluent treated surface soils				---------------mg L^{-1}---------------				
non	0-20	eff I treated ext.	8.1	87.7	0.2	254.7	101.5	153.2
non	0-20	eff II treated ext.	8.2	56.8	0.2	124.0	69.8	54.2
irri	0-20	eff I treated ext.	8.0	86.0	5.8	283.2	99.5	183.7
irri	0-20	eff II treated ext.	8.3	63.7	5.0	176.1	90.7	85.4
Lagoon effluent				---------------mg L^{-1}---------------				
--	--	eff I (0.2 μm)	7.7	103.0	0.1	346.6	167.4	179.2
--	--	eff II (0.2 μm)	8.3	84	0.1	230.8	142.6	88.2

† water ext = water extract; eff I treated ext = water extract of effluent-treated soils; eff I (0.2 μm) = effluent I filtered through 0.2 μm filter.

irrigated surface soil. Since the capacity test was conducted under nonlimiting NO_3^--N conditions, the higher rate of N_2O-N production suggests the presence of greater amounts of more readily available C in the irrigated surface soil. This was supported by the higher DOC concentrations in the irrigated soils (Table 5-1). Although not all of the DOC is available to denitrifiers, the significantly higher DOC concentrations for the irrigated surface soil suggested that along-term irrigation with C-rich dairy effluent resulted in accumulation of a large amount of available DOC to support denitrification. This long term role of organic carbon in promoting denitrification and development of a denitrifier population is also indicated by the higher denitrification rate and denitrification enzyme activity observed for the irrigated surface soil (Chapter 4).

Similar N_2O production for irrigated surface soil with and without NO_3^--N addition during the first three days indicated that the soil initially had sufficient NO_3^--N to support denitrification (Fig. 5-1). After 3 days, however, the NO_3^--N left in the soil could no longer sustain as high a denitrification rate as during the first three days. This initial lack of response to NO_3^--N additions has been reported in other NO_3^--N contaminated environments (Bradley et al.1992).

The much lower denitrification capacity of subsoils in both irrigated and non-irrigated areas was likely due to the lack of available organic carbon and/or small number of denitrifiers in the subsurface environment. In fact, DOC concentrations in the subsoils of the irrigated and non-irrigated areas were only 57 and 32% of those in the corresponding surface soils (Table 5-1). The amount of N_2O-N production in the subsoils was still measurable, but apparently limited by available organic carbon or microbial numbers.

Comparisons between the capacity and control treatments for the non-irrigated subsoils suggested that denitrification in the subsoils was also limited by NO_3^--N, as indicated by the increase in N_2O-N production upon addition of NO_3^--N to the subsoils (Fig. 5-1). There appeared to be sufficient DOC in the irrigated subsoil to support denitrification, but denitrification was limited by NO_3^-. Similar N_2O production rates for the first 7 days in the irrigated subsoil with and without added NO_3^- demonstrated that the irrigated subsoil could support denitrification at least for 7 days, even though the denitrification rates were low.

The relatively higher denitrification capacity for the non-irrigated subsoil compared to the irrigated subsoil (Fig. 5-1) may reflect the higher DOC concentrations in the non-irrigated subsoil (Table 5-1). Although the reason for higher DOC concentrations in the non-irrigated subsoil was not clear, the correlation between DOC and denitrification has been reported in a number of studies (Burford and Bremner 1975, McCarty and Bremner 1993).

Whereas denitrification capacity is a measure of the ability of a given soil to denitrify under the preexisting C conditions, the denitrification potential is a measure of the maximum amount of NO_3^--N a given soil can denitrify under optimal conditions. The response of both surface soils to combined NO_3^--N and glucose amendments suggested that denitrification in the surface soil was not only limited by NO_3^--N, but also by available organic C supply. This high denitrification potential also suggested that the surface soil in both non-irrigated and irrigated areas had potential to serve as a sink for NO_3^--N; however, such potential can rarely be fully expressed under natural conditions. The greater response of N_2O-N production to glucose than to NO_3^- suggested that limitation of the denitrification activity imposed by C availability was greater than that imposed by NO_3^--N availability in the surface soils.

Potential N_2O-N production decreased markedly in the subsoils of both irrigated and non-irrigated areas, indicating that the subsurface soils generally had smaller denitrifying populations than the surface soils (Yeomans et al. 1992). This low denitrifying population seems to be caused by the long term short supply of available organic C. The higher potential N_2O-N production in the non-irrigated subsoil after a 10 day incubation seemed to indicate that the this subsoil was somewhat more favorable for development of a denitrifying population, presumably because of the relatively higher DOC contents. This is contradictory, however, to the higher DOC contents found in the irrigated subsoils (Chapter 3). High spatial variability in C distribution may have created an uneven (patchy) distribution of denitrification activity (hot spots) and corresponding denitrifier populations. This higher denitrification potential in soils with lower ambient NO_3^--N concentrations has been previously reported for peat sediments of the Florida Everglades (Gordon et al. 1986).

Although the number of denitrifiers may decrease sharply with depth, the population existing in the subsoils could still support measurable denitrification activity. A recent survey at dairy farms in North Florida showed that denitrifying bacteria exceeding 100,000 colonies per 100 ml were commonly observed in shallow groundwater samples (ranging from 15 to 60 feet) (Andrews 1994). It is reasonable to assume that significant number of denitrifying bacteria are also present in the subsoils of the present study; however, the function of these bacteria is likely limited by available organic carbon.

The fact that both subsoils and non-irrigated surface soil showed a lag phase in denitrification activity following glucose amendment suggested that time was required for the denitrifying population to develop or at least for the existing population to reestablish full activity before high denitrification rates could be reached. These denitrifying bacteria initially present in the soil would served as a catalyst for the subsequent rapid growth or resumption of full activity for a bacterial population. Such an increase of denitrifying bacteria numbers or activity upon addition of C sources was also reported by Trudell et al. (1986).

The rate of N_2O production in the surface soils decreased after 7 days, suggesting that denitrification was limited by some factor (Fig. 5-2). The stoichiometric equation for denitrification is often typified as the following reaction, assuming glucose as the C source (Beauchamp et al. 1989):

$$5(CH_2O) + 4NO_3^- + 4H^+ \text{--->} 2N_2 + 5CO_2 + 7H_2O$$

Accordingly, 1 mole of glucose is needed for reduction of 0.8 moles of NO_3^-. This is equivalent to a C:N ratio of 12:11.2 in the reaction. Based on the above stoichiometry, the 20 mg of glucose C added to the 20 g of soil in the denitrification potential test could denitrify as much as 19 mg of NO_3^--N, assuming that all of the glucose C was used for denitrification. At the end of the 10 day incubation, however, only 3.6 mg of N_2O-N had been produced. This suggests that NO_3^--N was unlikely the limiting factor for the lower production rates after 7 days of incubation. Although NO_3^--N depletion has been found responsible for a plateau in N_2O production in other studies (Christensen et al. 1990), it is still possible that depletion of readily available organic carbon became alimiting factor. Although the amount of C added was enough to denitrify all of the NO_3^--N in the soil based on the stoichiometry mentioned above, the C can be used by all other heterotrophic organisms as well. The added C could thus be consumed much more quickly than NO_3^--N, resulting in earlier depletion than for NO_3^--N.

Dairy Effluent as a Carbon Source for Denitrification

It was hypothesized that the dairy lagoon effluent could serve as a C source for denitrification. This was supported by the strong stimulatory effect of effluent on denitrification in the surface soils (Fig. 5-3). The significantly higher N_2O production for effluent I-treated surface soils than the control clearly indicated that the effluent from the primary lagoon could supply a large amount of available C for denitrification. In fact, effluent I-treated surface soils produced about 1/3 of N_2O-N that glucose (potential) treatment produced. The surface soils treated with effluent II produced significantly less N_2O-N than the effluent I treatments, suggesting that there was less available C in effluent II than in effluent I. This was consistent with the low DOC concentrations in effluent II (Table 5-1). The lower availability of organic C in effluent

II could also be due to the poor quality of its C. It is likely that C availability would be reduced during aerobic decomposition in the retention pond. Higher N_2O production in the control for non-irrigated soils than for the irrigated soils cannot be explained at this time.

The high N_2O production in the effluent treated surface soils could have significant implications under field conditions. Although totally anaerobic conditions are rarely found in the field, irrigation with dairy lagoon effluent could create a favorable environment for denitrification not only by providing C, but also by increasing the microbial respiration, and thereby creating anaerobic "hot spots" in the surface soil. Christensen et al. (1990) found that increased denitrification was preceded by an increase in microbial respiration after amending the soils with particulate organic matter.

Denitrification in NO_3^- contaminated groundwater or aquifers has become of great interest because of the significant role it can play with respect to NO_3^--N removal. Many researchers, however, have reported that denitrification in subsoils is limited by available organic C (Starr and Gillham 1993, Yeomans et al. 1992). It is postulated that C in the effluent can stimulate subsoil/aquifer denitrification if transported to lower soil depths. The increased N_2O production in subsoils treated with water extracts of effluent-amended surface soils supportes this hypothesis. The much higher N_2O production in effluent I-extract treated subsoils than for the control suggestes that denitrification in subsoils can be stimulated by available organic C in the extract. The higher N_2O production in the non-irrigated subsoil, as stated previously, may also be an indication of larger initial microbial populations due to the higher DOC contents in those soil samples. The decrease in N_2O production rate for effluent I extract-treated subsoils suggests that the stimulatory effect of this C on denitrification was short lived, probably due to rapid decomposition of this C.

The N_2O production in subsoils treated with effluent II extracts was significantly less than those with effluent I treatment, suggesting reduced C availability in effluent II. However, the continuous increase in N_2O production even after 10 days suggested that C in the effluent II extracts might eventually have a sizable effect on denitrification in subsoils. This effect may be achieved through gradual buildup in the denitrifying population.

When water extracts for surface soils were added to the corresponding subsoils, increased N_2O production was observed in the irrigated subsoil but not in the non-irrigated subsoil (Fig. 5-5). Since the non-irrigated subsoil had higher initial denitrification potential, the subsequent effect of the water extract was less significant. Both higher C concentration in the irrigated surface soil and lower initial subsoil denitrification potential may have contributed to the higher response of irrigated subsoil to addition of the surface soil extract.

Summary and Conclusions

Organic C is an important factor regulating denitrification in soil environments. In this chapter, we studied the effect of organic carbon on denitrification in both surface and subsurface soils from the study area.

Nitrous oxide production in surface soils of both irrigated and non-irrigated areas showed significant response to NO_3^- addition, suggesting that denitrification under natural field conditions was limited by NO_3 supply. Long term irrigation with C-rich dairy lagoon effluent has resulted in more favorable conditions for denitrification in the irrigated surface soils, by providing both NO_3^--N and organic C. The denitrification capacity of subsoils was much lower than that of the corrsponding surface soils, due in part to a lower denitrifying population as result of a lack of available organic carbon.

Both irrigated and non-irrigated surface soils showed high denitrification potential, indicating that surface soils in the study area could potentially denitrify large quantities of NO_3^- when conditions are suitable. However, the maximum denitrification potential will not likely be found in the field, because optimal conditions are rarely found in field soils.

Subsoils showed lower denitrification potential than did surface soils, presumably due to lower initial denitrifying populations in the subsurface environment. The lag time in N_2O production provided evidence for the development of a viable denitrifying population upon addition of available organic carbon. The denitrification potential seemed to correlate well with DOC contents in subsoil.

The dairy lagoon effluent provided a large amount of available organic C for denitrification in surface soils. The lower stimulatory effect of effluent from a retention pond compared to effluent from a primary lagoon was likely due to both smaller quantities and availability of DOC because of the further decomposition of organic carbon during storage.

Soil solution constituents in effluent-treated surface soils could increase the denitrification rate in subsoils if transported to lower depths in the soil profile. Organic C in the soil solution seemed to be responsible for this effect. This effect of soil solution C levels on denitrification seemed to be in part dependent on the time for C to move to the subsoil. Aerobic respiration could consume most of the available C in the effluent when applied on the soil surface, if allowed to remain there for sufficient time during transpot. This was suggested by the lower effect of water extracts from surface soils on denitrification in subsoils.

CHAPTER 6
SUMMARY AND SYNTHESIS

Summary

Nitrogen fertilizer and animal manures are major sources of NO_3^- contamination for shallow groundwater in agricultural areas. Many dairy farmers in Florida use the wastewater produced in dairies for irrigating crop fields. This practice not only solves the problem of waste disposal, but also provides nutrients for crop production. The effluent often supplies enough nutrients so that no additional fertilizer is necessary for the irrigated field. However, excess application of dairy effluent could potentially cause nitrate contamination of shallow groundwater. In this dissertation, I (i) investigated the effect of effluent irrigation on both soil chemistry and groundwater quality in a poorly drained sandy soil irrigated with dairy lagoon effluent; (ii) determined the denitrification rates and discussed the regulators of denitrification in soil profiles of the study site; and (iii) studied the role of organic carbon in regulating denitrification for both surface and subsurface soils. The study was conducted on a spray field at the Dairy Research Unit of the University of Florida near Hague, FL. A summary of the major findings relative to the original questions and objectives of this study follows:

(1) What impact does effluent irrigation have on groundwater quality?

The effluent used for irrigation was high in organic N, organic C and ammonium-N, with alkaline pH. Irrigation with dairy effluent increased NO_3^--N concentrations in the shallow groundwater, exceeding the EPA drinking water standard of 10 mg NO_3^--N L^{-1}. Rapid conversion from NH_4^+-N and organic N in the effluent to NO_3^--N (N mineralization and nitrification) occurred in the surface soil following effluent irrigation. This conversion enhanced the leaching of NO_3^--N to the groundwater. Irrigation with dairy effluent did not increase NH_4^+-N concentrations or change groundwater pH, however. The DOC concentrations were also increased in lower portions of the irrigated soil, suggesting the transport of DOC from the upper soil profile.

(2) What is the extent of the impact and its relation to groundwater hydrology?

High NO_3^--N concentrations were observed in the area downgradient from the irrigated zone, but not to the edge of the field. This disappearance of NO_3^- within the field-edge suggests that NO_3^- contamination has been limited to a relatively small area to date. The distribution of NO_3^--N in the study area was closely related to groundwater hydrology. The study area can be divided into two distinct subareas topographically: a flat irrigated area and a relatively steep area between the irrigated zone and nearby riparian zone. Groundwater in the irrigated zone had slow lateral movement, due to small water head changes. This enhanced the accumulation of NO_3^--N. Hydraulic head difference between the irrigated field and the riparian zone created a driving force for groundwater flow, towards the riparian zone, therefore extending the NO_3^--N contamination plume beyond the irrigation boundary.

(3) What is the effect of effluent irrigation on soil chemistry?

Irrigation with dairy lagoon effluent also increased NO_3^--N concentrations in the soil profile, with the surface soil having higher NO_3^--N concentrations than the rest of the profile. The NO_3^- in the surface soil was subject to rapid downward leaching in this highly permeable sandy soil. Once reaching the groundwater, NO_3^--N accumulated due to the low rate of lateral movement of the groundwater and of nitrate removal by denitrification.

Irrigation with dairy lagoon effluent also increased the dissolved organic carbon (DOC) content of the soil profile. The measurable DOC transported as deep as 80 cm below the soil surface.

(4) What is the denitrification rate in this system and what are major regulators in the soil profile?

The ambient denitrification rates were low for the surface soil, and decreased with depth. They were consistent with rates for sandy soils as reported in the literature. Anaerobic denitrification rates were much higher than ambient rates in the surface soils, clearly suggesting that denitrification in the surface soils was limited by the aerobic conditions. Higher denitrification enzyme activity in the surface soils suggested that denitrification was also limited by available organic C, though to a lesser extent than by oxygen. The low denitrification rates and denitrification enzyme activities in the lower depths of the soil profile suggested that denitrification was limited by organic carbon availability. A long-term lack of available organic C in the subsoils resulted in low denitrifying populations.

(5) What role does denitrification play in protecting groundwater from nitrate contamination?

It is difficult to estimate the amount of N loss through denitrification, since neither totally aerobic nor totally anaerobic denitrification would likely occur under the field conditions. A more realistic scenario would be an intermediate rate between theses two extremes. Based on an assumption that anaerobic denitrification occurs an average once per week in this effluent-irrigated soil, as much as 118 kg N ha^{-1} yr^{-1} can be lost through denitrification. Besides uptake by crops, denitrification plays an important role in reducing NO_3^--N inputs into the shallow groundwater.

(6) What is the role of organic C in regulating denitrification in surface and subsurface soils?

Organic C in the dairy effluent can serve as an important C source for denitrification in surface soil, as evidenced by significant increases in denitrification upon addition of the effluent. Effluent-treated surface soil can also provide DOC for subsoils, thereby promoting denitrification in subsoils. However, the organic carbon seemed to be rather quickly decomposed in aerobic soil profiles. The DOC transported to the greater soil depths was more recalcitrant and apparently less available to denitrifiers. The high denitrification potential in subsoils indicated that denitrifying population existed in the subsurface environment, but their activity was depressed by a long-term lack of organic C.

Synthesis

Effluent Irrigation and Groundwater Contamination

The idea of using dairy effluent for irrigation is to properly dispose of wastewater produced and to efficiently recycle any nutrients in the effluent for plant production. However, this economically sound idea should also be evaluated in terms of environmental consequences. This study showed that effluent irrigation could result in significant accumulations of NO_3^--N in shallow groundwater. Nitrate contamination of groundwater poses a great threat to the most valuable drinking water resource in North Florida: the Floridan Aquifer. The sandy soil and shallow water table depth of this study site made it particularly susceptible to NO_3 contamination.

Whereas the depth of impact of NO_3 contamination below the water table is unknown, such contamination seemed to be limited within a small area. Groundwater hydrology is an important factor in determining the extent of nitrate contamination of the groundwater. The gentle slope in the area resulted in slow lateral movement of groundwater and a resultant long residence time. The NO_3^- disappearance in the groundwater downgradient from the irrigated zone was likely the result of denitrification. Because of reduced NO_3^- input from the top soil to the downgradient zone, even a low denitrification rate could remove a relatively large quantity of NO_3^- during a long residence time.

Denitrification and its Regulation

Denitrification has been studied for many years, primarily in agricultural surface soils where it is considered as an undesirable process. There is less information regarding the role of denitrification in NO_3^- removal from subsoils and NO_3^- contaminated groundwater systems. The results from this study showed that denitrification could play an important role in (a) reducing the amount of NO_3^--N input to the groundwater as a result of denitrifying NO_3^- in the surface soil; and (b) limiting the extent of nitrate contamination in groundwater. However, these roles can be severely limited by many factors in the soil environment. Denitrification in surface soils is limited primarily by O_2, and to a lesser extent, by available organic carbon. This was clearly supported by low ambient and high anaerobic denitrification rates, and by the fact that the highest denitrification enzyme activities were observed in the surface soils. Aerobic conditions likely prevail in the surface soil. However, anaerobic denitrification could still be a significant sink for NO_3^--N in the irrigated soil due to much higher rates, even though anaerobic conditions may persist for shorter time periods than aerobic conditions.

The limitation of O_2 on denitrification is not as great in subsoils, where it is often replaced by a lack of available organic C. It was hypothesized that effluent irrigation could increase the DOC levels in the subsoils and hence promote denitrification. I did find the transport of DOC through the soil profile to about the 80 cm depth, but DOC in the groundwater did not increase appreciably. The low denitrification rates in the subsoil and groundwater suggest that aerobic decomposition in the top soil may have consumed most of the available C. Any DOC transported to lower soil depths could also be more recalcitrant and thus less available to denitrifiers.

Future Research Needs

Some possible future research interests that arose from the present study are listed below:

(1) A more intensive study is needed to investigate the nature of NO_3^- transport to groundwater. The research should focus on the effect of quantity and frequency of irrigation on nitrate transport through soil profiles.

(2) It should be interesting to study N transformations once the effluent has reached the soil surface. Possible topics include the rates of each transformation processes, including mineralization, ammonification, nitrification and denitrification. The objective is to evaluate the role of each process in N removal from the system.

(3) The effect of irrigation with dairy effluent on soil and groundwater denitrification needs to be studied in more detail. Irrigation is an event that can affect the soil in many ways. Denitrification rates need to be determined with greater frequencies before and immediately following a irrigation or rainfall, along with the changes in chemical, physical and biological conditions during the events.

(4) Since organic C has to move through the soil column before reaching the groundwater, transformations and changes in the bioavailability of DOC in the soil profile also need to be further studied.

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BIOGRAPHICAL SKETCH

Shoucang Yan was born on April 10, 1963, in CangZhou, HeBei Province, China. He entered the HeBei Agricultural University in BaoDing where he received his Bachelor of Science degree in agronomy in July 1983. He then worked as a technician at the LouTai State Farm for one year before entering graduate school at Beijing Agricultural University in the Fall of 1984. He received his Master of Science degree in environmental soil science in the Fall of 1987. Upon graduation, he worked as a teaching assistant in the Soil Science Department of Beijing Agricultural University, where he taught laboratory courses and conducted field and laboratory research. He was admitted to the graduate school of the University of Florida in the Fall of 1989, and became a candidate for the degree of Doctor of Philosophy in the Department of Soil and Water Science in the fall of 1993.

Shoucang Yan is married to Liang Li and they have a wonderful four-year-old son, Clayton.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Donald A. Graetz

Donald A. Graetz, Chair
Professor of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Konda R. Reddy
Graduate Research Professor
of Soil and Water Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Brian L. McNeal
Professor of Soil and Water
Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

G. Ronnie Best
Scientist of Environmental
Engineering Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Yuch-Ping Hsieh
Professor of Wetland
Ecology, Florida A & M
University

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1995

Dean, College of
Agriculture

Dean, Graduate School